CHEMOTHERAPY AGAINST *STREPTOCOCCUS MUTANS*

THIJS SCHAEKEN

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(De horizontale lijn is op de achterkant verlengd.)

CHEMOTHERAPY AGAINST *STREPTOCOCCUS MUTANS*

CHEMOTHERAPIE TEGEN *STREPTOCOCCUS MUTANS*

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF DR. J H G.I GIESBERS VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN IN HET OPENBAAR TE VERDEDIGEN OP DONDERDAG 10 MEI 1984 DES NAMIDDAGS TE 4 UUR

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MATHIAS JOSEPH MARIA SCHAEKEN

GEBOREN TE WEERT

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PROMOTOR : PROF. DR. K.G. KÖNIG
CO-REFERENT: DR. J.S. VAN DER HOEVEN

Paranimfen: Hanny Lintsen
Henk de Jong

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Contents

I Introduction

The caries susceptibility of individuals is widely different (Bay and Ainamo, 1974; Truin *et al.*, 1984). In most patients caries can be prevented or arrested by the conventional preventive and restorative measures. In a minority of patients, however, this is not the case. The restorative treatment of patients with deep carious lesions or much secondary caries is difficult and leads to more complications, and as a consequence this small groups demands a disproportionally large part of the dental service.

The high caries activity of the risk patients can be associated with a diminished salivary flow - e.g. caused by the use of medicine or after radiation therapy in the head and neck region -, orthodontic treatment, the frequent intake of high amounts of sugar and a cariogenic plaque flora. These risk patients are in need of extra preventive help. A problem is however, that as long as the restorative treatment does not derange, the risk patients are difficult to recognize. Only patients in which the salivary glands are damaged after radiation therapy inevitably become high caries risk patients.

Caries activity measurement

In the past 25 years a great number of tests have been developed to predict or measure the caries activity: most of them, however, do not meet the requirements (Ellen, 1976; Newbrun, 1983). A relatively good indication for the prospective caries activity is the DMFS-count (Koch, 1970; Birkeland *et al.*, 1976) or the number of initial smooth surface lesions (Klock and Krasse, 1979). For individual cases this clinical criterium does not suffice, aside from the disadvantage that predictions can be made only when carious lesions have developed.

The microbiological tests that have been developed in recent years have proven to be more suitable. They are based on the measurement of the counts of *Lactobacillus* species and *Streptococcus mutans* in saliva or in dental plaque (Van der Hoeven, 1981; Newbrun, 1983).

The *Lactobacillus* test (Dentocult®, Orion Diagnostica, Helsinki, Finland; Larmas, 1975) is based on the number of lactobacilli in saliva. In subjects with a well maintained dentition - i.e. no open

cavities, no defective restoration margins - without prosthodontic or orthodontic devices, the number of lactobacilli in saliva corresponds with the degree of sugar intake. High *Lactobacillus* counts, above 10^5 CFU/ml saliva, indicate a high sugar intake.

The *S. mutans* count as a predictive value for caries activity is based on the relation of this bacterium with developing carious lesions (Bratthall, 1980). Elevated levels of *S. mutans* in plaque on a particular site of the teeth are often associated with a developing carious lesion (Shklair *et al.*, 1972; Loesche *et al.*, 1975a; Burt *et al.*, 1983). Subjects with a high caries activity have more sites infected with *S. mutans* than subjects with a low caries experience (Gibbons *et al.*, 1974) and patients with more than 10^6 CFU *S. mutans*/ml saliva develop more carious lesions than subjects with lower *S. mutans* counts (Klock and Krasse, 1979; Zickert *et al.*, 1983).

In the *S. mutans* test, the number of *S. mutans* cells in saliva (Klock and Krasse, 1979; Zickert *et al.*, 1982, 1983) or on a representative tooth surface (Burt *et al.*, 1983) is used to select caries susceptible subjects.

The predictive value of the *Lactobacillus* and *S. mutans* counts has a certain error: approximately 20 % of the subjects with high counts do not develop caries and likewise 20 % of the subjects with low counts do not remain free from new lesions (Krasse, 1976; Crossner, 1981). The error in these microbiological methods limits the prognostic value in the individual case.

When a combination of caries activity tests is used, the predictive value can be considerably enhanced. In a population without large numbers of retention sites such as open cavities, the *Lactobacillus* count, the *S. mutans* count and the number of initial smooth surface lesions was found to be a good combination (Klock and Krasse, 1979).

Preventive treatment of risk patients

In theory caries can be prevented by changing caries promoting food patterns, by making the tooth surface more resistant to bacterial acids and by transforming a cariogenic microflora into a non-cariogenic.

Changing the behaviour and food pattern of people has proven to be very difficult. In the explanations that have been given for the declining caries prevalence this factor is referred to only

occasionally (Marthaler, 1984). Yet, supported by the *Lactobacillus* counts, food counseling has given good results in individual cases (Krasse, 1976).

Of prime importance in the prevention of caries is the use of fluoride. The manner in which fluorides reduce dental caries is not fully understood, but most probably is related to making the tooth surface less soluble to the acids formed by the dental plaque flora.

In principle cariogenic plaque can be turned into a non-cariogenic plaque by antimicrobial treatment. The cariogenicity of the plaque microflora is presumably caused by a limited number of bacteria (Bowden *et al.*, 1984), of whom *S. mutans* is the most cariogenic (Hamada and Slade, 1980).

The antimicrobial treatment of *S. mutans* infections has been carried out with a variety of agents such as antibiotics - penicillin, furadroxyl, vancomycin, kanamycin -, disinfectants - iodine, chlorhexidine - and concentrated fluoride compounds - acidulated fluoride phosphate, stannous fluoride -. In clinical trials these agents have proven to be effective in caries prevention (reviewed by Loesche, 1982). Especially fluoride and chlorhexidine have received much attention.

The antimicrobial action of fluoride

The interaction of the fluoride ion with the toothsurface probably plays the crucial role in caries inhibition. Besides this, fluoride affects the carbohydrate metabolism in oral streptococci (Hamilton, 1977). Fluoride inhibits the enzyme enolase and thus the degradation of sugar into lactic acid. Indirectly the Phosphoenolpyruvate (PEP) dependent transport of sugar over the cell membrane (the Phospho Transferase System, PTS) is inhibited by fluoride.

Further, it is known that fluoride passes the cell membrane as HF and thus acts as a proton carrier. Dissociation of HF in the cell lowers the pH of the cytoplasma and reduces the pH difference across the cell membrane (Eisenberg *et al.*, 1980).

The inhibition of the bacterial metabolism is probably of minor importance for the caries preventive effect of fluoride. No differences have been found in the composition of the plaque flora of subjects living in areas with high levels of fluoride and subjects living in

areas with low fluoride levels (De Stoppelaar *et al.*, 1969; Kilian *et al.*, 1979b) or between subjects given fluoride supplements from birth and controls (Van Houte *et al.*, 1978). These observations suggest that the majority of the oral microflora is either resistant against fluoride or can adapt to fluoride. *S. mutans* species are known to adapt to fluoride concentrations in the millimolar range. Indeed, it has been shown that *S. mutans* in the dental plaque of gnotobiotic rats adapted to fluoride administered in the diet and in the drinking water (Van der Hoeven and Franken, 1984). The adaptability appeared from the fact that the adapted *S. mutans* plaque produced lactic acid when sugar and fluoride are applied simultaneously, while the unadapted *S. mutans* plaque does not produce lactic acid in the presence of fluoride.

In recent years the use of stannous fluoride, SnF_2, has gained more interest. The MBC of stannous fluoride is much lower than of sodium fluoride (Yoon and Berry, 1979; Mayhew and Brown, 1981; Maltz and Emilson, 1982). The extra antimicrobial effect of SnF_2 would stem from the stannous ion which reduces plaque acidogenicity (Opperman and Johansen, 1980) and plaque formation (Svatun, 1981) independent of fluoride ions (Ellingsen, Rölla and Svatun, 1982).

Clinical studies in which the effect of NaF and SnF_2 was compared, however, give contradictory results. Tinanoff *et al.* (1983) and Svanberg and Rölla (1982) found lower *S. mutans* levels in the SnF_2 group than in the NaF group. McHugh *et al.* (1983) did not see significant differences in the plaque composition after 1 year of SnF_2 or NaF rinsing with respect to *S. mutans*, *Veillonella*, total streptococci and total cultivable flora.

Concentrated fluoride compounds have a strong antimicrobial action. After a short-term intensive treatment, the initial plaque formation is suppressed, while the *S. mutans* counts are selectively reduced for a larger period of time (Loesche *et al.*, 1973, 1975b; Keene *et al.*, 1977; Kilian *et al.*, 1979a).

The antimicrobial action of chlorhexidine

Chlorhexidine is a basic molecule and within the range pH 4-9 di-cationic. It is adsorbed rapidly onto bacterial surfaces. At high concentrations (> 0.5 %) chlorhexidine is bacteriocide and acts as a

detergent by damaging the cell membrane and precipitating cytoplasmic components (Leach, 1977).

Chlorhexidine is inhibitory to yeasts and fungi and a wide spectrum of gram-positive and gram-negative oral bacteria (Hennessey, 1973). *In vitro S. mutans* is more susceptible to chlorhexidine than many other bacteria (Emilson, 1977).

After a short-term intensive treatment of the dentition with chlorhexidine *S. mutans in vivo* is suppressed for a long time while other bacterial species, and particulary *Streptococcus sanguis*, recover fast after the treatment (Emilson, 1981, Maltz *et al.*, 1981, Zickert *et al.*, 1982, Kristoffersson and Bratthall, 1982, Schaeken *et al.*, 1984). The strong antimicrobial action of chlorhexidine *in vivo* is most likely related to the fact that chlorhexidine is adsorbed to the teeth and mucosal surfaces and is subsequently released in bacteriostatic concentrations over a prolonged period of time (Gjermo *et al.*, 1974).

The mode of action of chlorhexidine at low concentrations is not precisely known. Possibly it is based on the inhibition of the Phospho Transferase System that is used by oral streptococci to transport sugars (Marsh *et al.*, 1983). *In vitro*, chlorhexidine inhibits the acid production by oral streptococci (Luoma *et al.*, 1972, Maltz-Turkienicz *et al.*, 1980) and *in vivo* the acidogenicity of dental plaque is reduced after rinsing with chlorhexidine (Opperman, 1979, Opperman and Gjermo, 1980).

Caries reduction by fluoride and chlorhexidine

The caries reducing action of fluoride is known for long and has been described extensively (reviews by Newbrun, 1978 and Murray and Rugg-Gunn, 1982). Fluoride supplementation of the drinking water inhibits the caries process on smooth and approximal surfaces better than in fissures and pits (Backer Dirks, 1974). The various fluoride compounds that are used in methods of application do not differ greatly in reducing caries. Thus the reduction of caries by NaF or SnF_2 is approximately the same in man (Newbrun, 1978, Murray and Rugg-Gunn, 1982) and in animals (Tinanoff and Camosci, 1984).

Due to the widespread use of fluoride few clinical studies have measured solely the effect of chlorhexidine. Experiments with dental students have produced inconclusive data on the caries reducing action

of chlorhexidine because the caries incidence in the control and the experimental groups was very low (Von der Fehr *et al.*, 1975; Johansen *et al.*, 1975; Emilson and Fornell, 1976). However, experimental caries, produced by frequent sucrose rinses could be strongly reduced by chlorhexidine rinses (Loe *et al.*, 1972) and in a study with school-children chlorhexidine rinses caused a 30-40 % reduction in caries incidence compared with control rinses (Okada, 1980).

In most studies on the caries inhibiting effect of chlorhexidine, the control and experimental groups were also treated with fluoride. In animal experiments this resulted in an additive anti-caries effect (Regolati *et al.*, 1974; Brayer *et al.*, 1977). Also in clinical studies the combination of chlorhexidine plus fluoride caused a greater caries inhibition than fluoride alone, although the effect was not always significant (Luoma *et al.*, 1978; Dolles and Gjermo, 1980; Zickert *et al.*, 1982). This may be due to the low caries incidence in the populations that were studied. In that case too many subjects do not develop caries at all, and extra preventive measures cannot have additional effect. In caries-active populations these studies would probably result in larger differences between the treatments. Indeed, it has been shown that in patients that had received radiation therapy in the head and neck region the combination of chlorhexidine plus fluoride inhibited significantly more caries than fluoride alone (Katz, 1982). Similar results were obtained by Zickert *et al.* (1982) in a study in teenagers: in risk-subjects, with *S. mutans* counts higher than 10^6 CFU/ml saliva, the combined chlorhexidine and fluoride was significantly more effective in inhibiting caries than fluoride alone. No such difference was found in the low-risk group with low salivary *S. mutans* counts.

The additive anti-caries effect in animals and in humans has been interpreted as evidence for chlorhexidine and fluoride having different sites of action (Marsh *et al.*, 1983).

Own investigations

The microflora of diseased tissues is often mono-specific or is dominated by a single microorganism (Alexander, 1971): in the case of caries this is nearly always *S. mutans*. However, there are carious lesions from which *S. mutans* cannot be isolated (Loesche and Straffon,

1979), while on the other hand high levels of *S. mutans* may be present without any sign of a carious lesion. These observations emphasize the importance of the other oral microorganisms in the caries process (Bowden *et al.*, 1984).

The normal plaque flora, the "basic" plaque (Marsh, 1980) generally includes many species and is characterized by homeostasis. The composition of such a microflora is, within certain boundaries, constant in time. All available niches in the plaque ecosystem are occupied and it is very difficult for alien microorganisms to colonize the plaque (Bowden *et al.*, 1979). The existance of this colonization resistance has been demonstrated in several clinical and animal experiments (Van der Hoeven, 1980).

Svanberg and Loesche (1977) implanted artificial fissures in subjects with high salivary levels of *S. mutans*. Empty fissures were rapidly colonized by *S. mutans* but when the fissure was filled with oral bacteria, *S. mutans* could not establish anymore.

The colonization of *S. mutans* in rats can also be retarded or inhibited. The colonization resistance of the dental plaque microflora in Osborne-Mendel rats (Animal Laboratory, University of Nijmegen) is low. Approximately 10^4 cells are required for the establishment of *S. mutans* OMZ176. Supplementation of the plaque microflora with biotypes of *Actinomyces viscosus* and *S. sanguis* increased the colonization resistance to a threshold dose of 10^{10} cells of OMZ176. Further, it was observed that the longer the delay in introducing *S. mutans* is, the poorer it established (Van der Hoeven, 1980) *S. sanguis* and *A. viscosus* are bacteria that compromise a large part of the basic plaque flora.

Based on the results from these clinical and animal experiments, we have conducted an experiment where we have tried to increase the colonization resistance of the human dental plaque against *S. mutans*. In volunteers *S. mutans* was first suppressed by a short intensive treatment with chlorhexidine and subsequently high numbers of *A. viscosus/naeslundii* and *S. sanguis* were applicated on the treated surfaces. It was observed that the increase of the *A. viscosus/naeslundii* population in the first 7 days following chlorhexidine treatment was the same in the group in which *A. viscosus* and *S. sanguis* were applicated as in the control group (unpublished results). To explain these disappointing results the experiments described in

chapter 2 were conducted. In the previous experiment the *A. viscosus/naeslundii* strains were applicated immediately after chemotherapy, and their colonization might have been affected by the chlorhexidine, retained after the antimicrobial treatment. Indeed, it was found that the initial colonization of *A. viscosus* was negatively affected after chlorhexidine application. Therefore, we have in subsequent experiments studied the effect of antimicrobial treatment on the kinetics of recolonization of important members of the oral microflora such as *S. mutans*, *S. sanguis* and *A. viscosus*. This was first done in a rat experiment (chapter 3) where the effect of chlorhexidine, iodine and 5,7-dichloro-8-hydroxyquinoline, DCHQ, was studied on the plaque composition. From clinical studies, chlorhexidine and iodine (Caufield and Gibbons, 1979) were known to strongly suppress *S. mutans*. DCHQ is an agent that *in vitro* strongly inhibits *S. mutans*, but has little effect on *A. viscosus* (Tanzer *et al.*, 1978).

In the clinical study described in chapter 4 chlorhexidine and iodine were applied onto well-localized spots on the dentition of volunteers, after which the kinetics of recolonization of *S. sanguis*, *S. mutans* and *A. viscosus* were recorded.

In order to achieve a long-lasting suppression of *S. mutans* the entire dentition was subsequently treated with chlorhexidine and stannous fluoride by using an intensive short-term high-dose regime (chapter 5). Despite a strong reduction of the salivary *S. mutans* counts the recolonization of *S. mutans* could not be postponed for more than 3 weeks. Therefore, the additional effect of daily rinsing with a low concentration of chlorhexidine was monitored.

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II The effect of Chlorhexidine on the Colonization of the Human and Rat Dentition by *Actinomyces viscosus*

M H de Jong, M J M Schaeken C W A van den Kieboom J S van der Hoeven

Department of Preventive and Community Dentistry University of Nijmegen, The Netherlands

Key Words. *Actinomyces viscosus* Chlorhexidine Plaque *Streptococcus mutans*

Abstract. The dentition of humans and rats was treated with a short term, high-dose application of chlorhexidine This strongly suppressed the indigenous microflora on the teeth At different time intervals after chlorhexidine application, a dominant member of the human plaque microflora, *Actinomyces viscosus*, was inoculated to investigate the effect of the retained chlorhexidine on its establishment It was found that until 72 h after chlorhexidine application the initial colonization of the molar teeth of rats by *A viscosus* Ny1S was affected by the retained chlorhexidine In humans *A viscosus* Ut3SR colonized the oral cavity better after chlorhexidine application than after professional cleaning Under these conditions, however, the inoculated *A viscosus* Ut3SR still made up only 0 1-1% of the total *A viscosus/naeslundii* population on the teeth Inoculation of humans with an unlabeled *A viscosus* strain, isolated from their own oral cavity, did not contribute to the reestablishment of the indigenous *A viscosus/naeslundii* population after chlorhexidine treatment

In a number of clinical studies chlorhexidine has been used as a selective agent to suppress *Streptococcus mutans* in dental plaque [*Schiott* et al , 1976, *Emilson* 1981, *Maltz* et al , 1981, *Schaeken* et al , 1983] Along with *S mutans* the indigenous plaque flora is also suppressed This must be considered as a potentially undesirable effect since colonization of the plaque by *S mutans* can be negatively affected by the indigenous microflora [*Svanberg and Loesche* 1977 *van der Hoeven and Rogers* 1979] Therefore, it seems desirable that the indigenous microflora is reestablished as soon as possible after chlorhexidine treatment It has, however, been shown that chlorhexidine has a long-term bacteriostatic effect in vivo due to its retention in the oral cavity [*Gjermo* et al , 1974, *Bonesvoll* 1978] We, therefore, wanted to investigate how chlorhexidine, retained after the stop of chemotherapy, influenced the recolonization of the teeth by *Actinomyces viscosus*, a dominant member of the indigenous microflora

In the present animal experiment, con

ventional rats were treated with chlorhexidine for 7 consecutive days At various time intervals after the last chlorhexidine application, the rats were inoculated with *A viscosus*, which was not present before in the microflora 1, 3 and 14 days after the inoculation, samples were taken to determine the level of the inoculated streptomycin (S) resistant *A viscosus* Ny1S In humans a streptomycin-rifamycin (SR) resistant *A viscosus* human strain was inoculated immediately after professional cleaning of the dentition, or 5 h after cleaning and application of a chlorhexidine gel Plaque and saliva samples were taken 7 and 14 days after the inoculation to determine the level of the SR-resistant *A viscosus* strain

Materials and Methods

Bacterial Strains

A viscosus Ny1S [*Beckers and van der Hoeven* 1982], a S resistant mutant of the rodent strain *A viscosus* Ny1 was used in the animal experiment In the clinical study *A viscosus* Ut3SR a SR resistant mutant of the human *A viscosus* strain Ut3 was used *A viscosus* Ut3SR is indistinguishable from its parent strain in its ecological behavior [*de Jong* et al , 1983]

Antibacterial Treatment

Chlorhexidine digluconate was obtained from ICI In the rat experiment a 5% solution of chlorhexidine digluconate was applied with a soft brush to the molar teeth as described before [*Schaeken* et al , 1984] Demineralized water was used as a placebo In the clinical study the dentition of the volunteers was cleaned by polishing the teeth with a rubber cup and pumice and interproximally with unwaxed dental floss (Johnson & Johnson) After cleaning, chlorhexidine digluconate (2 5 %) in carboxymethylcellulose gel (4%) was applied topically for 5 min with preformed disposable trays (Centrays®, Cooper Care, Inc)

Inocula

A viscosus was grown on TPY broth with 0 2 %, glucose, until the mid logarithmic growth phase (12 h) at 37 °C under a N_2 (90%), CO_2 (6%), H_2 (4%) atmosphere TPY broth contains 20 g trypticase peptone (BBL) and 10 g yeast extract (Difco) per liter of demineralized water The cultures were centrifuged for 10 min at 15,000 g and the pellet was dispersed in 1 10 volume of 0 85% (w v) NaCl The rats were inoculated with 100 µl of this cell suspension, applied under the tongue, using a micropipette (Boehringer) with a disposable tip The human volunteers were inoculated within 5 min after the preparation of the cell suspension, by washing their mouths vigorously with 10 ml of the cell suspension for 1 min

Animal Experiment

140 conventional Osborne Mendel rats, 37–42 days old, were distributed at random among 7 groups of 20 animals each After distribution the animals were fed a 516S diet [*Mikx* et al , 1972] ad libitum The animals were housed in cages with 3 or 4 animals each The experimental treatment of the animals is given in table 1 Chlorhexidine digluconate solution (5%) or demineralized water, used as a placebo, was applied for 7 consecutive days (table 1) After the last chlorhexidine application, the animals were inoculated with *A viscosus* Ny1S, at times as indicated in table 1 From each experimental group 6 or 7 animals were anesthetized with carbon dioxide and killed by decapitation, 24 and 72 h and 14 days after inoculation with *A viscosus* Groups 1 and 3 were sampled at the same time as groups 2 and 4 (table 1)

Bacteriological Samples The molars of the left lower jaw were extracted asceptically with a dental probe and were ground in a sterile mortar in 0 5 ml 0 85% (w/v) NaCl The suspension was transferred to a sterile tube and the mortar was rinsed with an additional 0 5 ml saline Pooled smooth surface and pooled fissure plaque was collected from the molars of the right lower jaw as described before [*Schaeken* et al , 1984] The samples were dispersed ultrasonically with a Kontes K881440 sonifier, provided with a microtip, for 30 s, at 0 °C, at maximal output 0 1 ml portions of suitable dilutions of the samples were plated on blood agar and TS S agar TS S agar contains 40 g trypticase soy agar (BBL) 10 g yeast extract (Difco), and 100 mg streptomycin (Gist Brocades, Delft, Netherlands) per liter of demineralized water The plates were incubated for 3 days (blood

Table 1 Experimental design of animal experiment

Experimental groups	Desinfectant	Inoculum	Time interval h
1	water	none	-
2	water	*A. viscosus*	1
3	chlorhexidine	none	-
4	chlorhexidine	*A. viscosus*	1
5	chlorhexidine	*A. viscosus*	6
6	chlorhexidine	*A. viscosus*	24
7	chlorhexidine	*A. viscosus*	72

The molar teeth of rats were treated for 7 consecutive days with chlorhexidine digluconate (5%) or water as a placebo. At different time intervals after the last chlorhexidine application *A. viscosus* Ny1S was inoculated.

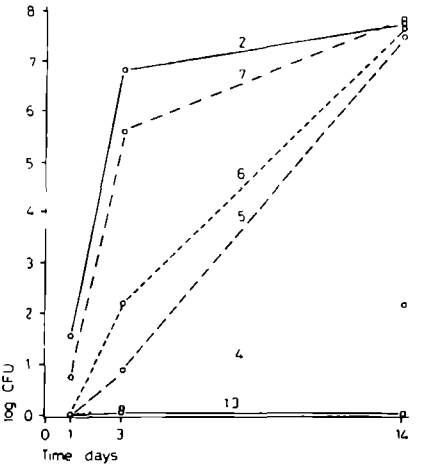

Fig. 1 The colonization of the molar teeth of rats by *A. viscosus* Ny1S after chlorhexidine application. *A. viscosus* Ny1S was inoculated at different time intervals after chlorhexidine application. The mean ^{10}log CFU of *A. viscosus* Ny1S on the extracted and ground molars of the left lower jaw, 1, 3 and 14 days after inoculation is given. Group 1 (——) control water treated, not inoculated; group 2 (——) control, water treated, inoculated after 1 h; group 3 (——) control, chlorhexidine treaed, not inoculated; group 4 ( ) chlorhexidine treated, inoculated after 1 h; group 5 (— —) chlorhexidine treated, inoculated after 6 h; group 6 (— — —) chlorhexidine treated, inoculated after 24 h; group 7 (— —) chlorhexidine treated, inoculated after 72 h.

agar) and 5 days (TS S agar) at 37 °C under an atmosphere of N_2 (90%), CO_2 (6%) and H_2 (4%). On blood agar the indigenous microflora was counted and on TS S agar *A. viscosus* Ny1S was counted.

Clinical experiment

The dentition of two groups of 7 volunteers was cleaned professionally with pumice and rubber cup. In the first group *A. viscosus* Ut3SR was inoculated immediately after professional cleaning. In the second group *A. viscosus* Ut3SR was inoculated 5 h after cleaning and chlorhexidine application. Samples were taken 7 and 14 days after inoculation with *A. viscosus*.

Bacteriological Samples. Pooled plaque samples were taken with a subdermal needle (12 × 0.04 mm) fitted in a needle holder from the following sites: fissure plaque from the occlusal surfaces of 35, 36 and 37, and smooth surface plaque from the lingual surface of 36 and 37. Pooled approximal plaque was collected with sterile unwaxed dental floss (Johnson & Johnson) from the distal surfaces of 35 and 36 and from the mesial surfaces of 36 and 37.

After sampling, the needles and the floss with the adherent plaque were placed in vials with 1 ml of reduced transport fluid [*Loesche* et al. 1972]. 5 ml of unstimulated saliva was collected in a small vial. Plaque and saliva samples were dispersed ultrasonically for 20 s with a Kontes K 881440 sonifier, provided with a microtip, at 0 °C, at maximal output. 0.1-ml portions of suitable dilutions of the samples were plated on blood agar, CNAC 20 agar and TS SR agar. The plates were incubated for 5 days under the conditions described above. On blood agar the total cultivable flora was counted. *A. viscosus* and *A. naeslundii* were counted on CNAC 20 agar [*Ellen and Balcerzak-Raczkowski* 1975, *Schaeken* et al. 1983]. The two *Actinomyces* species cannot be distinguished from each other on colonical morphology on

Table II Implantation of *A viscosus* in humans

Sample	*A viscosus* Ut3SR		*A viscosus/naeslundii*	
	A	B	A	B
Smooth surface plaque	0 19 ± -	3 61 ± 1 98	6 24 ± 1 04	5 89 ± 2 36
Approximal surfaces plaque	0 81 ± 1 06	2 68 ± 1 65	6 19 ± 0 66	5 62 ± 1 20
Fissure plaque	0 34 ±	1 39 ± 1 50	3 79 ± 2 69	3 76 ± 1 22
Saliva	2 26 ± 1 34	4 51 ± 1 10	7 02 ± 2 37	6 56 ± 1 31

In group A *A viscosus* Ut3SR was inoculated immediately after professional cleaning In group B *A viscosus* Ut3SR was inoculated 5 h after professional cleaning and chlorhexidine application (2 5% gel for 5 min)
Inoculated *A viscosus* Ut3SR population mean ¹⁰log CFU ± SD 7 days after inoculation
Indigenous *A viscosus naeslundii* population mean ¹⁰log CFU ± SD 7 days after inoculation

this medium and were counted together *A viscosus* Ut3SR was counted on TS SR agar TS SR agar has the same composition as TS S agar and is supplemented with 25 mg rifamycin l (Le Petit SA, Seclin)

For calculation purposes the number of colony forming units (CFU) was transformed to the ^{10}log CFU [*Caufield and Gibbons* 1979]

Results

Animal Study

In rats colonization of the teeth by *A viscosus* Ny1S (fig 1) was retarded by the preceding treatment with chlorhexidine The retardation was dependent on the time elapsed between the termination of chemotherapy and the inoculation of *A viscosus* The effect of chlorhexidine was still observable when *A viscosus* was inoculated as long as 72 h after the end of chemotherapy (fig 1) In the group where *A viscosus* was inoculated 1 h after the last application of chlorhexidine, the organism finally established in only 2 of 6 rats The effect of chlorhexidine on the rate of increase of *A viscosus* was the same in smooth surface and fissure plaque samples (results not shown) as in ground molar samples (fig 1)

Clinical Study

When *A viscosus* Ut3SR was inoculated after dentition cleaning, it colonized the oral cavity very poorly Its proportion remained very low compared to the total population of *A viscosus/naeslundii* biotypes (table II) Following chlorhexidine application in addition to cleaning *A viscosus* Ut3SR established considerably better in the human oral cavity However, the population of *A viscosus* Ut3SR still did not exceed the level of 0 1–1% of the total *A viscosus/naeslundii* population (table II) In both experimental groups the inoculated *A viscosus* Ut3SR was always found in saliva samples

Discussion

Gjermo et al [1974] suggested that the plaque-inhibiting effect of chlorhexidine in humans was largely due to a bacteriostatic effect caused by the retention and

slow release of the drug in the oral cavity [*Rolla* et al., 1971], rather than on its initial bactericidal effect. Without questioning the importance of retention of chlorhexidine for its plaque-inhibiting effect, *Roberts and Addy* [1981] have suggested that the drug is not truly desorbed, but lost from the oral cavity due to the normal turnover of biological surfaces. In this study the indigenous microflora was suppressed first by a short-term, high-dose (5%) chlorhexidine application. It has been shown before that in this way long-term suppression of *S. mutans* from the oral cavity of rats could be obtained, presumably because *S. mutans* was eliminated [*Schaeken* et al., 1984]. After the stop of chemotherapy, the effect of the residual chlorhexidine on the developing microflora was investigated by inoculating *A. viscosus*.

In the rat experiment *A. viscosus* was not present in the microflora prior to inoculation. Inhibition of the colonization of the rodent strain *A. viscosus* Ny1S was only observed in the group of rats inoculated 1 h after the stop of chemotherapy. In this group of rats only 2 of 6 animals were infected (fig. 1). A bacteriostatic effect could initially be seen in the other experimental groups, where the increase of *A. viscosus* was retarded in the first 3 days following inoculation (fig. 1). This shows that the retained chlorhexidine was biologically active for at least 72 h. Whether the biological activity is due to a true desorbtion of the drug and subsequent readsorbtion by newly inoculated cells, or to the attachment of newly inoculated cells to biological surfaces saturated with chlorhexidine cannot be differentiated. 2 weeks after inoculation the population levels of *A. viscosus* (fig. 1), *S. bovis* and the total cultivable flora (results not shown) were indistinguishable from those in the control group.

Inoculation of the human *A. viscosus* strain Ut3SR (SR-resistant) in volunteers after professional cleaning resulted in very poor colonization of the oral cavity (table II). *Bamman* et al. [1978] reported that S-resistant *S. mutans* strains colonized poorly in rats. For *A. viscosus* Ut3SR, however, it has been shown before that the strain is indistinguishable from its parent strain in its ecological behavior in rats [*de Jong* et al., 1983]. Therefore the SR resistance does not seem to be the cause of the poor colonization. It seems more likely that a competitive interaction with the remaining indigenous microflora prevented the successful colonization of the oral cavity [*Beckers and van der Hoeven*, 1982].

In the second group of volunteers, *A. viscosus* Ut3SR was inoculated 5 h after cleansing and chlorhexidine application. It was assumed that after 5 h the residual chlorhexidine would only have a bacteriostatic effect (fig. 1). Indeed no inhibition of the colonization by the retained chlorhexidine was observed since *A. viscosus* Ut3SR established in all volunteers. The better establishment of *A. viscosus* Ut3SR following chlorhexidine treatment is likely to be explained by depression of the indigenous microflora [*Schaeken*, unpublished results]. It can be expected that colonization of an inoculated *A. viscosus* strain is counteracted by the indigenous population.

7 days after chlorhexidine application, the indigenous *A. viscosus/naeslundii* population had reached its steady-state level (table II), indistinguishable from the population level 7 days (table II) or 14

days (results not shown) after cleaning At this time the inoculated strain *A viscosus* Ut3SR contributed only a small part of the total *A viscosus/naeslundii* population in the chlorhexidine treated group of volunteers Therefore, it seems that inoculation of *A viscosus* after chlorhexidine treatment in humans does not stimulate the recovery of the total *A viscosus/naeslundii* population This was confirmed in an experiment where localized areas on the dentition of volunteers were inoculated with a large dose of an indigenous *A viscosus* strain isolated from their own oral cavity It was observed that the increase of the *A viscosus/naeslundii* population in the first 7 days following chlorhexidine treatment was the same in the inoculated group as in the non inoculated control (results not shown) Maybe removal of the retained chlorhexidine immediately after application [*Gjermo* et al , 1974] will enable us to inoculate *A viscosus* more successfully in the human oral cavity

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M H de Jong
Department of Preventive and
Community Dentistry
University of Nijmegen
Philips van Leydenlaan 25
NL-6800 HB Nijmegen (The Netherlands)

III Effects of Chlorhexidine, Iodine, and 5,7-Dichloro-8-Hydroxyquinoline on the Bacterial Composition of Rat Plaque in vivo

M J M Schaeken C W A van den Kieboom H C M Franken M H de Jong J S van der Hoeven

Department of Preventive and Community Dentistry University of Nijmegen The Netherlands

Key Words. *Actinomyces viscosus* Chlorhexidine Dichlorohydroxyquinoline Iodine Plaque *Streptococcus mutans*

Abstract. The effects of short term high-dose therapies with chlorhexidine, iodine, or 5,7-dichloro 8-hydroxyquinoline (DCHQ) on the microbial composition of dental plaque in conventional rats were compared The antiseptics were applied to the molars on 7 consecutive days Smooth surface and fissure plaque samples were analyzed 1, 3, and 10 days after the end of therapy All antiseptics exerted a stronger effect on the microflora on smooth surfaces than in fissures Chlorhexidine, but not iodine or DCHQ, eliminated *Streptococcus mutans* from plaque Other species, such as *Actinomyces viscosus*, were suppressed but not eliminated Microbial species that were affected, but not eliminated by chemotherapy were found to return to their original level The data support the use of chlorhexidine for clinical suppression of *S mutans*

A number of antiseptics, among which chlorhexidine, iodine, and 8-hydroxyquinoline derivatives, have been reported to change the microbial composition of dental plaque Studies on the effect of chlorhexidine on the microbial composition of dental plaque in humans [*Emilson* 1981, *Rindom Schiott*, 1973] and in animals [*Emilson and Westergren* 1979, *Emilson* et al , 1981a, b] showed that *Streptococcus sanguis* was generally less susceptible to chlorhexidine treatment than *Streptococcus mutans* or *Actinomyces viscosus* These in vivo results were in accordance with studies on the in vitro susceptibilities of *S mutans*, *S sanguis*, and *A viscosus* to chlorhexidine [*Maltz-Turkienicz* et al , 1980, *Emilson*, 1977] Iodine was reported to reduce *S mutans* levels in humans at approximal sites for variable periods of time [*Gibbons* et al , 1974, *Caufield and Gibbons*, 1979, *Newbrun* et al , 1980] In in vitro studies *S mutans* and *Actinomyces* species were shown to be more sensitive to iodine than *S sanguis* [*Tanzer* et al , 1977, *Maltz-Turkienicz* et al , 1980] *Tanzer* et al [1978]showed that 5,7-dichloro-8-hydroxyquinoline (DCHQ) was highly selective in

inhibiting the in vitro growth of *S. mutans* as compared to *S. sanguis* and oral *Actinomyces* species. The in vivo effect of DCHQ on dental plaque has not been reported so far.

In this experiment the changes taking place in the microbial composition of dental plaque 1–10 days after the end of a short term high dose treatment were studied. Three antiseptics, i.e. chlorhexidine, iodine and DCHQ, were compared with each other for their ability to eliminate *S. mutans* selectively from dental plaque. As will be shown in this paper the microbial composition of dental plaque 10 days rather than 1 day after the end of treatment is an indication of the effect of the antiseptic. It was observed by some authors that chlorhexidine [*Emilson* et al., 1981a] and iodine [*Caufield* et al., 1981] had an anti caries effect only on smooth surfaces, but not in fissures. However other authors reported that both fissure and smooth surface caries were reduced by chlorhexidine [*Regolati* et al., 1974; *Muhlemann* 1973]. Therefore, in our study, separate plaque samples from smooth surfaces and fissures were taken in addition to samples consisting of extracted and ground molars.

Materials and Methods

Antiseptics

Chlorhexidine digluconate was obtained as a 20% (w/v) aqueous solution from ICI, London, England; iodine and potassium iodide were obtained from Merck, Darmstadt, FRG; and DCHQ was obtained from Aldrich Europe, Beerse, Belgium. Antiseptics were applied as chlorhexidine digluconate 5% solution (w/v) in demineralized water; iodine 2% iodine and 2% potassium iodide (w/v) in demineralized water; DCHQ 2% solution (w/v) in polyethyleneglycol 300. DCHQ was dissolved at 60°C and kept at this temperature until application to prevent recrystallization. Demineralized water was applied to the animals from the control group.

Microorganisms

A. viscosus Ny1, *S. sanguis* Ny101 and *S. mutans* T S (streptomycin resistant) were used in this study and were kept lyophilized in stock. All microorganisms were as used in an earlier study [*Van der Hoeven and Rogers* 1979].

Media

TPY broth contained 20 g trypticase peptone (BBL) and 10 g yeast extract (Difco) per litre of demineralized water. TS agar contained 40 g trypticase soy agar (BBL) and 10 g yeast extract (Difco) per litre of demineralized water. TSS agar had the same composition as TS agar but 100 mg of streptomycin (Gist brocades, Delft, Netherlands) was added per litre. Blood agar contained 25 g brain heart infusion (Difco), 10 g bactopeptone (Difco), 1 g potassium nitrate (Merck) and 25 g of agar (Difco) per litre of demineralized water. After sterilization and cooling to 56°C, 100 ml of defibrinated sheep blood was added per litre.

Inocula

All bacterial strains were grown in TPY broth at 37°C under a N (91%), CO (5%), H (4%) atmosphere. Inocula were prepared from cultures in their late logarithmic growth phase (*A. viscosus* 36 h, *S. sanguis* and *S. mutans* 24 h). The cells were dispersed ultrasonically in the growth medium by treating the cultures for 30 s at 0°C with a Kontes cell disruptor K 881440 provided with a microtip at maximum output. The animals were inoculated with 100 µl of the sonified cultures applied with a Boehringer micropipette under the tongue.

Animal Experiment

96 conventional Osborne Mendel rats, 34–36 days old from eight litters were distributed at random among four groups of 24 animals each (day 0). The animals were caged in groups of 4 animals each. After inoculation the animals were given diet 516 S containing 16% sucrose [*Mikx* et al., 1975]. On day 2 the animals were inoculated with *A. viscosus* Ny1 and *S. sanguis* Ny101 and on day 4 with *S. mutans* T S in order to create the so called SPF Ny flora with 5·10⁸ *S. mutans* T [*Van der Hoeven and Rogers*

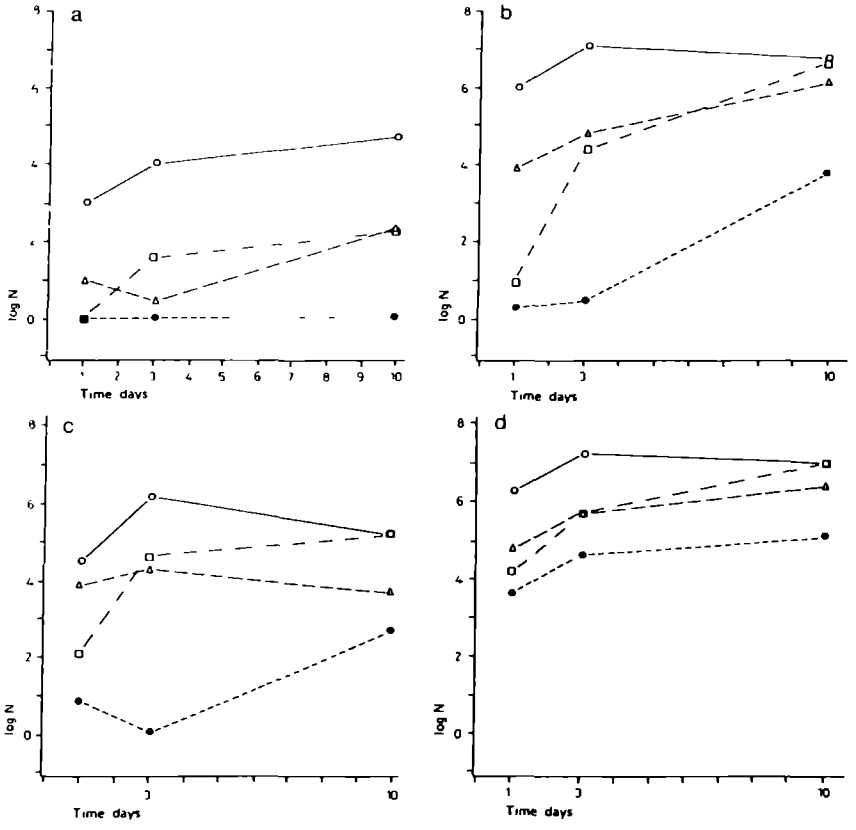

Fig. 1. Mean ^{10}log number of CFU (log N) in smooth surface plaque from the 1st molar ○ = H_2O (control), ● = 5% chlorhexidine, □ = 2% I-, △ = 2% DCHQ **a** *S mutans*, **b** *A viscosus*, **c** *S bovis* **d** Total viable counts

1979] The flora was then allowed to stabilize till day 14, when the application of the antiseptics started The antiseptics were applied with a soft brush on the molars of the right and left upper and lower jaws for 10 s once a day for 1 week 8 animals from each experimental group were killed and sampled on days 21 24, 30 re, 1, 3, or 10 days after the last application of antiseptics

Bacteriological Sampling

The molars of the left lower jaw were extracted asceptically with a dental probe and were ground in a sterile mortar in 0 5 ml 0 85% (w/v) saline The suspension was transferred to a sterile tube, and the mortar was rinsed with an additional 0 5 ml saline Smooth surface plaque was collected with a dental probe from the buccal, lingual, and mesial surface of the 1st molar, and a pooled fissure plaque sample

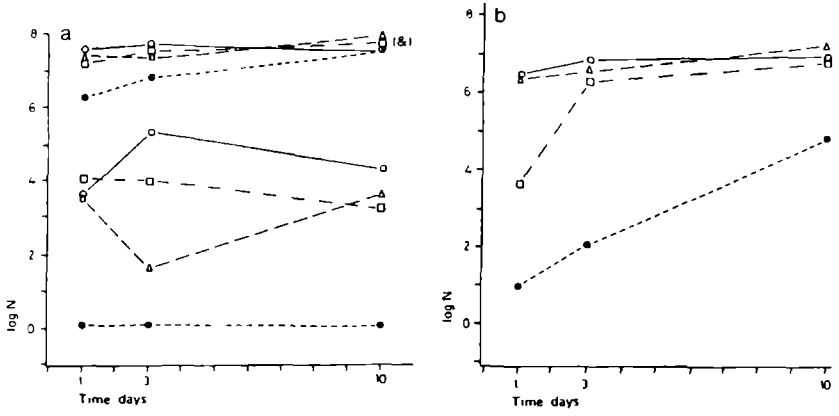

Fig. 2. Mean ^{10}log number of CFU (log N) in plaque from the split fissures of the 2nd and 3rd molar ○ = H_2O (control), ● = 5% chlorhexidine, □ = 2% I, △ = 2% DCHQ **a** *S mutans*; upper part (&) *total viable counts* **b** *A viscosus.*

was taken from split central fissures of the 2nd and 3rd molars of the right lower jaw Care was taken to collect all available plaque from these sites; even when no visible plaque was present on the smooth surfaces, the surface was wiped several times with the dental probe Plaque samples were transferred into 100 µl sterile 0 85% (w/v) saline All plaque samples were treated separately and dispersed with a Kontes sonifier as described above Suitable dilutions of the samples were plated on blood agar, TS agar, and TSS agar The TS and blood agar plates were incubated for 72 h at 37 °C in an atmosphere of 91% N_2, 4% H_2, and 5% CO_2 The TSS plates were incubated for 96 h under the same conditions On blood agar *A viscosus* Nyl, *S mutans* T_2S, and *Streptococcus bovis* (*S bovis* is part of the indigenous flora of the Osborne-Mendel rats from the Nijmegen breeding laboratory) could easily be distinguished from each other and from other components of the flora and were counted separately The total cultivable flora was also counted on blood agar On TS agar, *A viscosus* Nyl and *S mutans* T_2S could be distinguished and counted On TSS agar only *S mutans* T_2S was counted

For statistical analyses the numbers of colony-forming units (CFU) were transformed to the ^{10}log CFU [*Caufield and Gibbons*, 1979] Analyses of variance on the ^{10}log CFU were performed with a SAS general linear model program (SAS Institute, Cary, N C , USA)

Results

Topical application of chlorhexidine for 7 days eliminated *S. mutans* T_2S from smooth surfaces (fig. 1), fissures (fig. 2), and also from the ground molar samples (results not shown). Iodine and DCHQ did not significantly decrease the number of CFU of *S. mutans*, as compared to the control, in the fissures or in the ground molar samples. On the smooth surfaces *S. mutans* was found to be significantly reduced by both agents on days 1 and 3 after therapy ($p < 0.05$; Scheffé's test), but not on day 10 (fig. 1).

From figure 1 it can be seen that species that were suppressed, but not eliminated, by an antiseptic returned to the level found in the control group For all species and in all samples chlorhexidine exerted the strongest effect (fig 1, 2) 10 days after the end of chemotherapy, the total viable counts or CFU of separate species in the iodine or DCHQ groups were no longer significantly different from those in the control group However, in all samples of the chlorhexidine group, the numbers of *S mutans* and *A viscosus* Ny1 were still significantly lower than in the control group ($p < 0.05$, Scheffe's test) In all four groups *S sanguis* Ny101 was present at too low a level for a meaningful analysis.

Discussion

The initial colonization of tooth surfaces in gnotobiotic rats by *S mutans* and *A viscosus* was investigated by *Beckers and Van der Hoeven* [1982] They found that after a lag phase a period of rapid increase in the population occurred, until the rate of increase declined as the bacterial populations approached a stationary level. From figures 1 and 2 it can be seen that the same kinetics applied to bacterial populations that were affected, but not eliminated, by the treatment with antiseptics These populations returned to the stationary level observed in the control group in a similar way Therefore, in clinical studies, the effects of short-term therapies on the microbial populations in plaque should be evaluated after sufficient time has elapsed for the population to attain a stationary level As compared to the iodine group, the growth of *A viscosus* (fig 1, 2) and *S boris* (fig 1) in the chlorhexidine group was retarded This is likely to be due to retention of chlorhexidine in the mouth [*Rolla* et al, 1971, *Gjermo* et al, 1974] It was found in this study that all antiseptics exerted a stronger effect on the microbial populations in the more easily accessible smooth surface plaque than on the populations in the fissures (fig 1, 2)

Caufield [1981] and *Caufield* et al. [1981] observed that in rats, iodine had an anticaries effect mainly on smooth surfaces This agrees with the observation in our study that iodine affected only the *S mutans* population in smooth surface plaque, but not in fissure plaque These observations suggest that iodine does not penetrate deeply into the fissures Variable results were reported for the time during which an iodine treatment could suppress the *S mutans* levels in approximal plaque in humans [*Gibbons* et al., 1974, *Caufield and Gibbons*, 1979] It was also noticed that repeated treatments were necessary to maintain a low level of *S mutans* at approximal sites [*Newbrun* et al, 1980] This agrees with our observation that bacterial populations not eliminated from the plaque return to their original level The return of *S mutans* at a particular site to its original level can occur either by outgrowth of a few remaining viable cells at that site or by reinfection via the saliva The first process presumably results in a much faster return of *S mutans* than the latter process

Although DCHQ was reported to have a highly selective effect against *S mutans* in vitro [*Tanzer* et al, 1978], this component was found to be almost inactive in vivo, presumably because of its low solubility in water [*Tikus*, 1974].

A viscosus, *S bovis*, *S mutans* and the total viable counts were much more strongly inhibited by chlorhexidine than by any other antiseptic used in this study. In fissures chlorhexidine was the only compound with a significant effect on the microbial populations. Furthermore, chlorhexidine was highly selective as it was the only antiseptic that eliminated *S mutans* from the plaque on both smooth surfaces and in fissures. This agrees with the findings of *Regolati* et al. [1974] and *Muhlemann* [1973] who showed that fissure as well as smooth surface caries could be reduced by chlorhexidine treatment. Different results obtained by other authors [*Emilson* et al., 1981a], who only found reduction of smooth surface caries, must be explained by assuming that under the conditions of dosage or application of chlorhexidine that they used, *S mutans* was not eliminated from the fissures.

Our results support the use of chlorhexidine for clinical suppression of *S mutans*. However, *S mutans* must be wholly eliminated from the mouth or when reduced, but not eliminated, must be prevented from returning to its original level. The latter could perhaps be accomplished by competitive exclusion of *S mutans* from the plaque by other species [*Van der Hoeven and Rogers* 1979].

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M H de Jong,
Department of Preventive and
Community Dentistry,
University of Nijmegen,
Nijmegen (The Netherlands)

Caries Res 816

IV Effect of Chlorhexidine and Iodine on the Composition of the Human Dental Plaque Flora

M J M Schaeken M H de Jong H C M Franken J S van der Hoeven

Department of Preventive and Community Dentistry University of Nijmegen The Netherlands

Key Words. *Actinomyces viscosus* Chlorhexidine Iodine *Streptococcus mutans* *Streptococcus sanguis*

Abstract. Localized areas of the dentition in human volunteers were treated once with chlorhexidine or iodine Plaque samples taken from the experimental surfaces were analyzed for the number of *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus* and the total viable counts Chlorhexidine and iodine strongly suppressed *S mutans* and *A viscosus*, but *S sanguis* was much less affected *A viscosus* returned to its original level within 7 days after chemotherapy *S mutans* returned much slower to its pretreatment level In fissures and restoration margins, *S mutans* was still significantly suppressed 21 days after chlorhexidine application

In a considerable number of studies a significant association of *Streptococcus mutans* with human dental caries has been found [*Newbrun*, 1979] Various agents such as antibiotics, antiseptics and fluorides have been tested to suppress *S mutans* in dental plaque [*Loesche* 1976] Little attention has been paid to the possible detrimental effects of these agents on bacterial species other than *S mutans* in the plaque Yet, suppression of the indigenous microflora must be considered as an undesirable effect in the chemotherapy of *S mutans* infections because the colonization of the plaque by *S mutans* can be negatively affected by the indigenous flora Such an interaction was shown by *Svanberg and Loesche* [1977] who found that *S mutans* could easily colonize an empty fissure, but failed to establish once the fissure was occupied by other bacteria Further, *Beckers and van der Hoeven* [1982a, b] found that the growth rate of *S mutans* in the early stage of dental plaque formation was considerably suppressed by other bacteria, and experiments by *van der Hoeven and Rogers* [1979] suggested that *Actinomyces viscosus* and *Streptococcus sanguis* could increase the colonization resistance of dental plaque in rats against *S mutans* Thus, for clinical use there is reason to look for agents that selectively suppress *S*

mutans and have little effect on other plaque bacteria.

From in vitro studies it is known that substances such as vancomycin and kanamycin [*Newbrun* et al., 1976], iodine [*Tanzer* et al., 1977], 5,7-dichloro-8-hydroxyquinoline [*Tanzer* et al., 1978] and chlorhexidine [*Maltz-Turkienicz* et al., 1980] exert a greater effect on *S. mutans* than on *S. sanguis* and *A. viscosus*. Clinical tests showed that vancomycin [*DePaola* et al., 1974], chlorhexidine [*Emilson*, 1981] and iodine [*Gibbons* et al., 1974; *Caufield and Gibbons*, 1979; *Newbrun* et al., 1980] were effective against *S. mutans*. However, chlorhexidine [*Emilson* et al., 1982] or iodine [*Newbrun* et al., 1980] also suppressed *A. viscosus*. *S. sanguis* seems to be less affected by chlorhexidine as its proportion increased after chlorhexidine application [*Emilson and Fornell*, 1976; *Emilson*, 1981].

In this study, a number of well-defined smooth-surface and occlusal areas within the same mouth were treated once with a high dose of chlorhexidine or iodine. The rest of the dentition was not treated with these agents. In this way, the effects of a single application of these two antiseptics on the plaque microflora could be studied as well as the kinetics of recolonization of the treated surfaces by *S. mutans*, *S. sanguis* and *A. viscosus*.

Materials and Methods

Participants

7 volunteers, students in social sciences between 19 and 27 years of age, participated in this study. The DMFS scores ranged from 11 to 75 but none of the subjects had gross carious lesions (i.e. $D_{3,4} = 0$). Dental prophylaxis or information on diet or oral hygiene were not given before or during the study.

In each subject, 10 well-defined areas on smooth surfaces and 10 well-defined areas in fissures or restoration margins were selected for sampling. Sites without detectable levels of *S. mutans* were discarded, the remaining sites served as experimental areas. In this way a total number of 75 sites, 36 on smooth surfaces and 39 in fissures or restoration margins were selected for a longitudinal study.

Treatment

In each subject the experimental areas, 3–7 on smooth surfaces and 4–7 in fissures/restoration margins, were randomly assigned to three groups. The surfaces in group I served as a control and were treated for 5 min with a placebo gel, consisting of 4% carboxymethylcellulose. The surfaces in group II were treated for 5 min with 5% chlorhexidine digluconate in carboxymethylcellulose gel and the surfaces in group III for 2 min with a solution of 2% I_2 in 2% KI in 53% glycerin [*Caufield and Gibbons* 1979]. Each experimental group consisted of 25 areas, 12 on smooth surfaces and 13 in fissures or restoration margins. The experimental reas in each subject were treated as follows: the surfaces were rinsed with water, dried and isolated with cotton rolls prior to application of the appropriate antiseptic or placebo gel. Care was taken to prevent contact of the agents with neighboring surfaces or saliva. After the application, the antiseptics were carefully removed with cotton rolls before the subjects rinsed with water.

Sample Collection

Samples were taken 28 days, 14 days, and immediately before chemotherapy in order to obtain the 'baseline' microbial composition of each site, and subsequently 2 days, 7 days and 21 days after the chemotherapy (fig. 1). At the beginning of each session 3 ml of saliva was collected. Salivary flow was stimulated by chewing on a piece of plastic. Prior to the plaque sampling, the adherent saliva on the surfaces was removed by water using a water spray. The plaque was then removed with a subdermal needle (12 × 0.4 mm) fitted in a needle-holder. The needle with the adherent plaque was transferred into a vial with 1 ml of reduced transport fluid [*Loesche* et al., 1972].

Bacteriological Procedures

Plaque and saliva samples were homogenized by ultrasonic dispersion for 20 s at 0°C using a Kontes

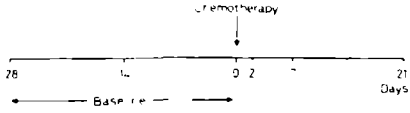

Fig. 1. Outline of the experimental design

cell disruptor K-881440 provided with a microtip 100 µl portions of appropriate dilutions of these samples were then plated onto blood agar, sucrose-agar and CNAC-20 agar All plates were incubated at 37 °C in a 91% N_2, 5% CO_2, 4% H_2 atmosphere for 5 days

On blood agar the total cultivatable flora was counted CNAC-20 agar [*Ellen and Balcerzak-Raczkowski*, 1975] is a selective and elective medium for *A viscosus* and *A naeslundii* The two *Actinomyces* species cannot be distinguished from each other on this medium on colonial morphology and were counted together Sucrose agar [*de Stoppelaar* et al , 1967] is a selective and elective medium for *S mutans*, *S sanguis* and polysaccharide-producing strains of *S mitior* *S mutans* can be distinguished from the other two species on the basis of colonial morphology and was counted separately. *S sanguis* and the polysaccharide-producing strains of *S mitior* were counted together

In cases of doubt, colonies from CNAC-20 or sucrose agar were isolated on blood agar Isolates from CNAC-20 were checked for cellular morphology with phase-contrast microscopy and isolates from sucrose-agar were tested for their ability to ferment sucrose, mannitol and sorbitol, to produce ammonia from arginine and to hydrolyze esculin

The bacteriological counts were ^{10}log transformed prior to statistical analysis The data were subjected to an univariate analysis of variance with repeated measures [*Winer*, 1971], individual comparisons were done with the methods developed by *Scheffé* [1961] for each of the counted bacterial species as well as for the total cultivatable flora.

Results

In fissures and restoration margins *S. mutans* was significantly reduced 2 days after treatment with chlorhexidine or iodine ($p = 0.001$ and 0.001, respectively, Scheffé) (fig. 2a) Chlorhexidine had a much stronger effect on the *S. mutans* population than iodine. 21 days after treatment the *S. mutans* counts in the chlorhexidine group were still significantly lower than the *S. mutans* counts in the control group ($p = 0.03$, Scheffé), while the *S. mutans* counts in the iodine group

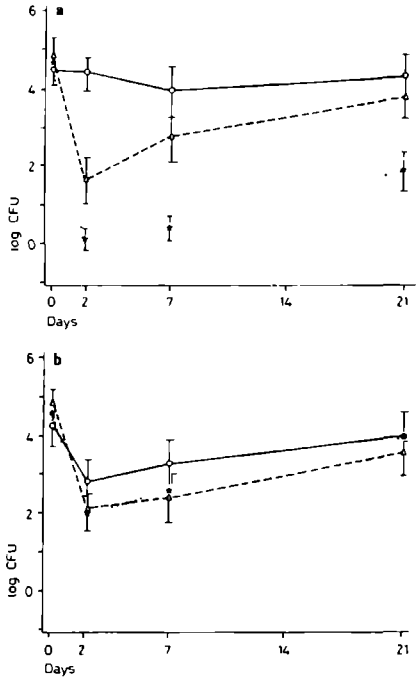

Fig. 2. a Mean ^{10}log number of *S mutans* counts in plaque from fissures and/or restoration margins (n = 13) O——O = control group, ★ ★ = chlorhexidine group, Δ- - - -Δ = iodine group b Mean ^{10}log number of *S mutans* counts in plaque from smooth surfaces (n = 12) (Symbols as in figure 2a)

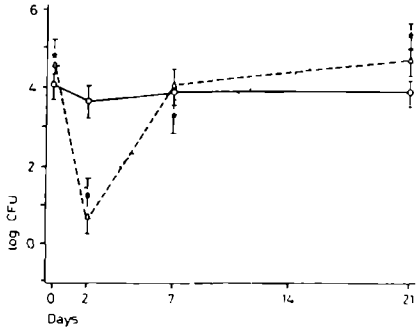

Fig. 3. Mean ^{10}log number of *A viscosus/naeslundii* counts in plaque from fissures and/or restoration margins (n = 13) O———O = control group, ★ ★ = chlorhexidine group, Δ- - - -Δ = iodine group

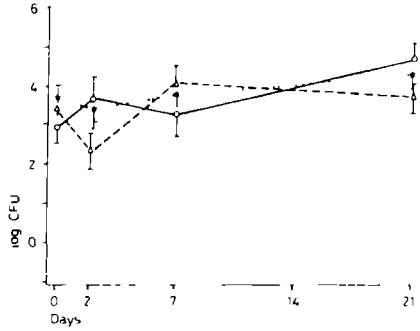

Fig. 4. Mean ^{10}log number of *S sanguis/mitior* counts in plaque from smooth surfaces (n = 12) O—O = control group, ★ ★ = chlorhexidine group, Δ- - - -Δ = iodine group

and the control group were no longer significantly different at 7 days after application ($p = 0.08$, Scheffé).

On smooth surfaces the *S mutans* counts in both the chlorhexidine and iodine group were not significantly reduced as compared to the control group. This was probably due to a drop in the *S. mutans* counts observed after placebo treatment (fig. 2b). In fissures and restoration margins no placebo effect was observed.

2 days after chemotherapy *A viscosus/naeslundii* in fissures and restoration margins was strongly suppressed by either chlorhexidine or iodine ($p = 0.001$, 0.001, respectively, Scheffé) (fig. 3). On smooth surfaces *A viscosus/naeslundii* was also strongly suppressed, but due to the placebo effect this was not statistically significant. In contrast to *S mutans*, *A. viscosus/naeslundii* had returned to its original level within 7 days after chemotherapy, both in fissures and restoration margins (fig. 3) and on smooth surfaces. The *S. sanguis/mitior* population on smooth surfaces (fig. 4) or in fissures or restoration margins was virtually not affected by the chemotherapy. As compared to the control, no significant reduction was observed in the number of total colony-forming units after chlorhexidine or iodine application, except for fissures and restoration margins 2 days after chlorhexidine application ($p = 0.04$, Scheffé) (results not shown).

The mean salivary concentration of *S. mutans* was 1.6×10^6 CFU/ml (individual means ranged from less than 10^4 to $4 \cdot 10^7$ CFU/ml), of *S. sanguis/mitior* $7.3 \cdot 10^6$ CFU/ml (individual means ranged from $4 \cdot 10^5$ to $3 \cdot 10^7$ CFU/ml) and of *A viscosus/naeslundii* $1.6 \cdot 10^7$ CFU/ml (individual means ranged from $6 \cdot 10^6$ to $4 \cdot 10^7$ CFU/ml). No significant differences in these mean values were seen in the samples taken before or after chemotherapy. However, in subjects with mean sali-

vary levels of *S mutans* above 10^6 CFU/ml, *S mutans* reappeared sooner on the treated surfaces than in subjects with lower salivary levels of *S mutans*

Discussion

In several clinical studies it has been demonstrated that *S mutans* can be suppressed by topical applications of chlorhexidine [*Emilson*, 1981, *Kristoffersson and Bratthall* 1982] or iodine [*Caufield and Gibbons*, 1979, *Newbrun* et al, 1980] Most of the studies have not dealt with the kinetics of recolonization by S *mutans* after chemotherapy and in just a few were the effects of chemotherapy on bacteria other than *S mutans* recorded

In the present experiment it was found that after a single application of chlorhexidine, *S mutans* was suppressed for more than 3 weeks, whereas the duration of the suppression caused by iodine was much shorter. This was in accordance with observations in rats [*Schaeken* et al, 1983] where chlorhexidine also more effectively suppressed *S mutans* than iodine It seems that the more persistent reduction of *S mutans* by iodine as found by *Caufield and Gibbons* [1979] must be ascribed to the repeated application of iodine in contrast to the single application that was used in our study

In the control group a significant reduction of *S mutans* was observed on the smooth surfaces following the application of the placebo gel Yet, we could not detect any inhibitory activity of placebo gel against *S mutans* in vitro It seems likely that the drop in *S mutans* counts was due to the frequent removal of plaque from the same sites The relatively short time intervals of 2 and 5 days between subsequent samplings might not be sufficient for *S mutans* to reach the original level Indeed, it has been shown in rats that at 5 days after inoculation, *S mutans* is still in a phase of logarithmic increase and far from a stationary level [*Beckers and van der Hoeven*, 1982b] The results further show that the effect of sampling was more pronounced for *S mutans* on smooth surfaces than in the fissures This reflects the better accessibility of the smooth surfaces for quantitative removal of the plaque The finding that *S mutans* reappeared sooner after chemotherapy in individuals with high salivary *S mutans* counts supports the view that this organism can be transmitted within the mouth via the saliva

It can be expected that the concentrated antiseptics suppressed other bacteria than *S mutans* Indeed, in this as well as other studies, *A viscosus* was found to be reduced after iodine or chlorhexidine treatment (fig 3) [*Newbrun* et al, 1980, *Emilson* et al, 1982] The low level of *S mutans* and *A viscosus* observed on day 2 after chemotherapy indicated that these 2 organisms were equally susceptible to the concentrated antiseptics The prolonged reduction of *S mutans* but not of *A viscosus* in fissures and restoration margins is likely to be ascribed to a different mechanism

Beckers and van der Hoeven [1982b] have described a competitive interaction between *S mutans* and the resident microflora As consequence the growth rate of *S mutans* after inoculation in conventinal rats is much slower than after inoculation in gnotobiotic rats (doubling time t_d was 5 h in conventional rats compared to 1 1 h

in gnotobiotic rats) *A viscosus* is hardly affected by the indigenous microflora as its growth rate in both types of rats is approximately the same ($t_{d\ conv}$ = 3 1 h, $t_{d\ gnoto}$ = 2 8 h) Further, *Svanberg and Loesche* [1977] have found that *S mutans* colonized an empty fissure within 1 day, but failed to establish once the fissure was occupied by other bacteria The findings in our experiment suggest that following antimicrobial therapy a competitive interaction between *S mutans* and the indigenous microflora occurred, while *A viscosus* was hardly affected

The recolonization of *S mutans* is probably retarded by bacteria that are only slightly or not affected by the antiseptic agent In our experiment the *S sanguis* counts (fig 4) and the total CFU were hardly affected by chemotherapy *Emilson* et al [1982] also observed that after chlorhexidine treatment in rats, *A viscosus* was suppressed strongly while *S sanguis* was not affected The small effect of chemotherapy on *S sanguis* is most likely related to its relative insensibility to chlorhexidine or iodine [*Emilson*, 1977, *Maltz-Turkienicz* et al , 1980]

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Dr M J M Schaeken,
Department of Preventive and Community Dentistry,
University of Nijmegen,
Nijmegen (The Netherlands)

V Effects of Highly concentrated stannous fluoride and chlorhexidine regimes on the Human Dental Plaque Flora

M.J.M. Schaeken, M.H. de Jong, H.C.M. Franken, J.S. van der Hoeven
Department of Preventive and Community Dentistry, University of Nijmegen, Nijmegen, The Netherlands

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ABSTRACT

The aim of this study was to determine the effect of an intensive antimicrobial treatment on the number of *Streptococcus mutans*, *Streptococcus sanguis*, *Actinomyces viscosus* and the total Colony Forming Units (CFU) in plaque.

The dentition of human volunteers was treated with either chlorhexidine (5 %) or stannous fluoride (8 %). Following local treatment with chlorhexidine, selected volunteers rinsed daily for 7 or 49 days with chlorhexidine solution (0.2 %) while another group flossed daily for 7 days with dental floss impregnated with chlorhexidine. On days 1, 7, 21, 35 and 49 after the local applications saliva samples and plaque samples from fissures, smooth surfaces and approximal areas, were taken.

Chlorhexidine and stannous fluoride suppressed *S. mutans* and *A. viscosus* on all surfaces and in saliva. *S. mutans* on tooth surfaces was suppressed for approximately 7 days and reached the baseline level at day 21. *A. viscosus* was suppressed for more than 7 days on the teeth. *S. sanguis* and the total CFU had regained the baseline level within 7 days on all surfaces and in saliva. Rinsing or flossing with chlorhexidine suppressed *S. mutans* for the time that these supplements were used.

Brushing for 7 days with chlorhexidinegel (1 %) without a preceding intensive chlorhexidine treatment had virtually no effect on *S. mutans* in approximal areas and in saliva, but suppressed *S. mutans* in fissures and on smooth surfaces.

In a previous study we have shown that spot application of chlorhexidine or iodine strongly suppresses *Streptococcus mutans* and *Actinomyces viscosus*. It was found that *A. viscosus* recovered from chemotherapy within 7 days while *S. mutans* needed considerably more time to re-establish, especially in subjects with low salivary counts of *S. mutans* (Schaeken *et al.*, 1984). This latter observation corresponded with results of Svanberg and Loesche (1977) and Duchin and van Houte (1978).

S. mutans can be suppressed by short-term intensive treatment with chlorhexidine (Emilson, 1981; Maltz, Zickert and Krasse, 1981; Zickert, Emilson and Krasse, 1982) or stannous fluoride (Keene, Shklair and Mickel, 1977). The success of these treatments, however, is only temporarily and within a few weeks *S. mutans* regains its original number in saliva and on the teeth. This indicates that there must be "reservoirs" that are hardly or not affected by chemotherapy and from which *S. mutans* recolonizes the dentition. Alternatively the mouth may become infected with new *S. mutans*.

In this study we have tried to eliminate *S. mutans* from the mouth by an intensive short-term treatment with stannous fluoride (SnF_2) or chlorhexidine. As the application of these agents was restricted to the dentition, potential *S. mutans* reservoirs on soft tissues (van Houte, 1976) remain unaffected. In addition to the local application we have, therefore, determined the effect of supplementary rinsing with a chlorhexidine solution and flossing with chlorhexidine impregnated dental floss.

The recolonization of *S. mutans* can be retarded or prevented by other members of the oral microbial flora (Svanberg and Loesche, 1977; van der Hoeven and Rogers, 1979; Beckers and van der Hoeven, 1982; Schaeken *et al.*, 1984). For this reason the effect of chemotherapy on *A. viscosus*, *Streptococcus sanguis* and the total cultivable flora was also recorded.

Participants and treatments

38 Subjects, students in social sciences between 20 and 33 years old participated in this study. The DMFS scores ranged from 7 to 89 but none of the subjects had gross carious lesions. The subjects were distributed ad random over 7 experimental groups (table) and were treated as indicated.

group	*n*	*treatment*
I	*6*	*placebo gel (control group)*
II	*7*	*chlorhexidine gel (chex. gel)*
III	*5*	*stannous fluoride solution*
IV	*5*	*chlorhexidine gel + 49 days rinsing with chex. solution*
V	*5*	*chlorhexidine gel + 7 days rinsing with chex. solution*
VI	*5*	*chlorhexidine gel + 7 days flossing with chex impregnated floss*
VII	*5*	*brushing at home for 7 days with chlorhexidine gel*

The subjects in group I served as a control and were treated with a placebo gel consisting of 4 % carboxymethyl cellulose. The subjects in group II, IV, V and VI were treated with a 5 % chlorhexidine digluconate (ICI, England) in 4 % carboxymethyl cellulose gel. The subjects in group VII brushed their teeth with a 1 % chlorhexidine digluconate in 4 % carboxymethyl cellulose gel and the subjects in group II were treated with a freshly prepared solution of 8 % stannous fluoride (SnF_2) (Merck, BRD) in demineralized water. The bitter taste of the chlorhexidine gel was masked with a few drops of peppermint oil. Prior to the treatment, the dentition of the subjects was cleaned with pumice and with unwaxed dental floss. Then, in each approximal area a drop of gel or SnF_2 solution was flossed interdentally and subsequently the respective gel or stannous fluoride solution was applied onto the dentition three times for 3 minutes with intervals of 5 minutes between each treatment. The gels were applied in preformed disposable trays (Centrays®, Pacemaker Corp.) simultaneously to upper and lower teeth. During the SnF_2 application the upper or lower dentition was isolated with cotton rolls, to avoid contact of the solution with the mucosa, and the SnF_2 solution was applied continuously to the teeth with cotton pellets. The subjects were allowed to rinse with tap water between the

subsequent applications. After the application of the chlorhexidine gel the subjects in group IV rinsed for 49 days and those in group V for 7 days, twice daily for 1 minute with 10 ml of a 0.2 % chlorhexidine solution (Hibident®, ICI). The subjects in group VI were instructed to floss their teeth once a day with unwaxed dental floss that had been impregnated for 24 hours with a 5 % chlorhexidine digluconate solution. Brushing with chlorhexidine gel (group VII) was done at home for 7 days once daily for 5 minutes.

Sample collection

In each subject 3 smooth surface areas, 3 fissures, 3 approximal areas and 3 sulci were selected for longitudinal sampling. Samples were collected before the dental prophylaxis and treatment, in order to obtain the baseline microbial composition, and subsequently 1, 7 and 21 days after the gel or stannous fluoride treatment. In group VI (chex gel + 7 days chex. floss) samples were taken 7, 14 and 28 days after the gel treatment. In the control group and in group IV (chex. gel + 49 days rinsing chex. solution) additional samples were taken 35 and 49 days after the gel treatment. In 3 subjects from group II (chlorhexidine gel) and in 3 subjects from group V (chex. gel + 7 days rinsing with chex. solution) plaque samples were taken from the attached gingiva (between tooth 13 and 17), from the palate (along the median line from the papilla incisiva to the foveolae palatinae), from the cheek (along a line from the orifice of the glandula parotis to the corner of the mouth) and from the tongue (along the central groove from papillae vallatae to the apex of the tongue). At the beginning of each session 3 ml of paraffin stimulated saliva were collected. Prior to plaque sampling the adherent saliva on the surface was removed by water spray. The plaque from fissures and smooth surfaces was then collected with a subdermal needle (12 x 0,4 mm) fitted in a needle holder; plaque from the approximal areas was collected with unwaxed dental floss (Johnson & Johnson®) and from the sulci with an extirpation needle (Maillefer®, fine). Plaque samples from the mucosal surfaces were taken with small cilindrical cotton pellets (Roeko, Bausch®) fitted in a college pincet. The pooled plaque from the fissures, the smooth surfaces, the approximal areas and the sulci and the plaque from the

mucosal surfaces was transferred into a vial with 1 ml of Reduced Transport Fluid (Loesche, Hockett and Syed, 1972).

Bacteriological procedures

Plaque and saliva samples were homogenized by ultrasonic dispersion for 20 s at 0 ^{o}C using a Kontes cell disruptor K-881440 provided with a microtip. 100-µl portions of appropriate dilutions of these samples were plated onto blood agar, TYCBS-agar and CNAC-20 agar. All plates were incubated at 37 ^{o}C in a 91 % N_2, 5 % CO_2, 4 % H_2 atmosphere for 5 days. On blood agar the total cultivable flora and *S. sanguis* were counted. CNAC-20 agar (Ellen and Balcerzak-Rackowski, 1975) is a selective and elective medium for *A. viscosus* and *A. naeslundii*. The two *Actinomyces* species cannot be distinguished from each other on this medium on colonial morphology and were counted together. TYCBS (van Palenstein Helderman *et al.* 1983) is a selective medium for *S. mutans* containing bacitracin and sucrose as selective agents.

Statistical procedures

The bacteriological counts were ^{10}log transformed prior to statistical analysis in order to homogenize the variances. The data were subjected to an univariate analysis of variance with repeated measures for each of the counted bacterial species as well as for the total cultivable flora (Winer, 1971). Unless otherwise indicated the comparisons were made between the control group and an experimental group and tested with the method developed by Dunnett (Winer, 1971).

The chlorhexidine treatment was generally well accepted by the subjects. On 1 day after the treatment 4 of the 22 subjects who received a local application complained of a painful gingiva upon toothbrushing. Clinically this was manifested by erosive plaques on the attached gingiva (Flotra *et al.* 1971). The discomfort subsided within 2-3 days. In 3 of the 5 subjects who had rinsed for 49 days with the chlorhexidine solution the brown stain had to be removed from the teeth at the end of the experiment. The application of chlorhexidine required 20-25 minutes per individual. Stannous fluoride application needed twice as much time because this agent could not be applied simultaneously on the upper and lower teeth.

Brushing with chlorhexidine gel for 7 days had virtually no effect on the *S. mutans* population in approximal areas and in saliva (results not shown). The suppression in fissures and on smooth surfaces (results not shown) was statistically not significant.

The application of chlorhexidine gel (group II) and stannous fluoride (group III) strongly suppressed *S. mutans* on day 1 in approximal areas ($P < 0.01$, fig. 1a), on smooth surfaces ($P < 0.05$, results not shown) and in fissures ($P < 0.01$, fig. 1b). On day 7 the reduction of *S. mutans* was no longer statistically significant. When the local application was followed by rinsing with a low concentrated chlorhexidine solution, or by flossing with chlorhexidine impregnated dental floss, *S. mutans* was suppressed for the time that these supplements were used (fig 1a,b,c). Rinsing with the chlorhexidine solution for 49 days (group IV) significantly suppressed *S. mutans* for the entire experimental period in fissures (day 49. $P < 0.01$) and for 35 days on approximal surfaces (day 35 $P < 0.05$).

When the chlorhexidine solution or floss was used for 7 days, *S. mutans* was suppressed during this period (day 7 $P < 0.01$, fig. 1a,b). The organism soon recovered after termination of chlorhexidine usage. The suppression of *S. mutans* by rinsing or flossing was not restricted to the smooth and approximal surfaces respectively, but occurred on other tooth surfaces as well as in saliva (results of rinsing shown in fig 1a,b,c, results for flossing not shown).

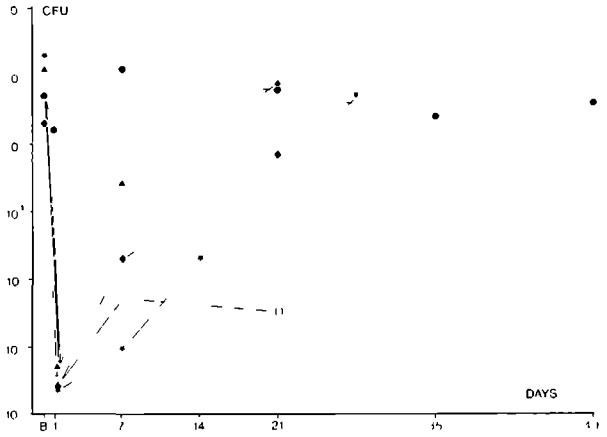

fig. 1a: *S. mutans* in approximal areas
- ● control (group I; n=6)
- ▲ application chlorhexidine gel (group II; n=7)
- ★ application chlorhexidine gel + 7 days chlorhexidine impregnated floss (group VI; n=5)
- ♦ application SnF_2 solution (group III, n=5)
- □ application chlorhexidine gel + 49 days chlorhexidine rinses (group IV, n=5)
- ▽ 7 days brushing with chlorhexidine gel (group VII, n=5)

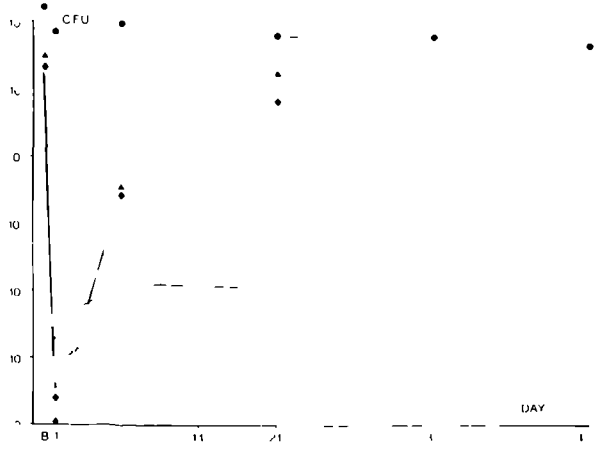

fig. 1b: *S. mutans* in fissures, same legend as fig. 1a

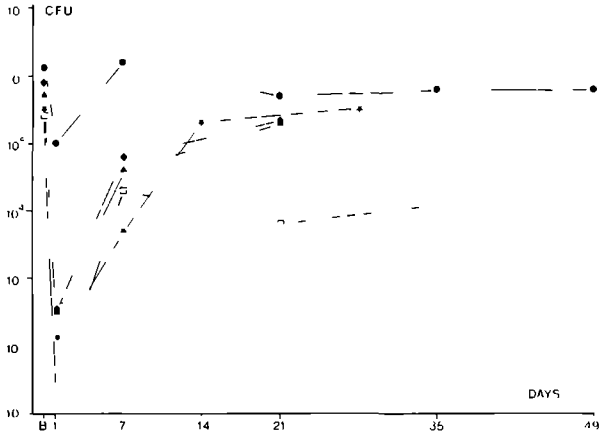

fig. 1c· *S. mutans* in saliva, same legend as fig. 1a

S. mutans was isolated from all mucosal surfaces and sulcul areas. Compared to the baseline level the *S. mutans* populations on all these sites were strongly suppressed on day 1 after chemotherapy ($P < 0.05$, Student's t-test, fig 1d).

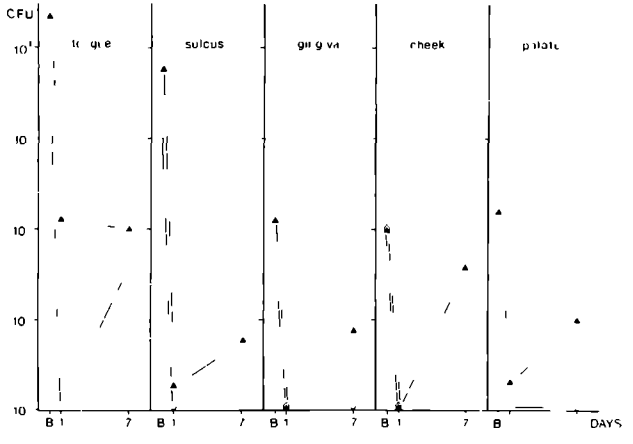

fig. 1d *S. mutans* in sulcus and on mucosal surfaces
▲ application chlorhexidine gel (group II, N=3)
◇ application chlorhexidine gel + 7 days chlorhexidine rinses (group V, n=3)

The *A. viscosus/naeslundii* population on tooth surfaces was strongly suppressed for at least 7 days following chlorhexidine gel application. The suppression of *A. viscosus/naeslundii* by SnF_2 was statistically not significant. The additional chlorhexidine rinses after the local chlorhexidine treatment (group IV, 49 days rinsing) suppressed *A. viscosus/naeslundii* during the entire experimental period in fissures (day 49: $P < 0.01$; fig. 2) and for more than 21 days on smooth surfaces (day 21: $P < 0.01$). The use of chlorhexidine rinses or -floss for 7 days had virtually no effect on the *A. viscosus/naeslundii* counts on the tooth surfaces or in saliva at day 21 (results not shown).

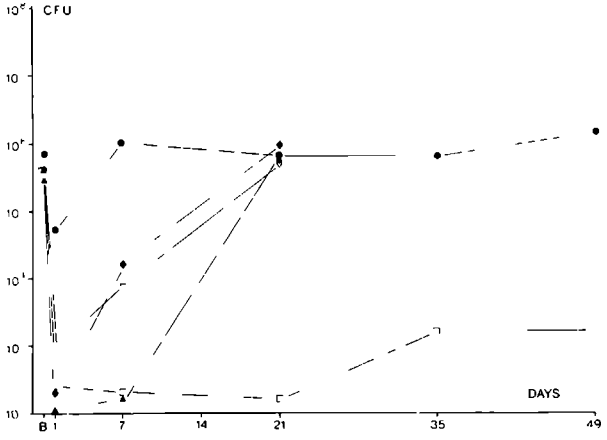

fig. 2: *A. viscosus/naeslundii* in fissures, same legend as fig 1a.

S. sanguis was strongly suppressed by chlorhexidine on smooth surfaces ($P < 0.05$; fig. 3) and in fissures 1 day after the local application. Within 1 week after the treatments *S. sanguis* had returned to the original level on all surfaces. Rinsing with chlorhexidine after the local application did not affect the population during the rest of the experimental period (fig. 3).

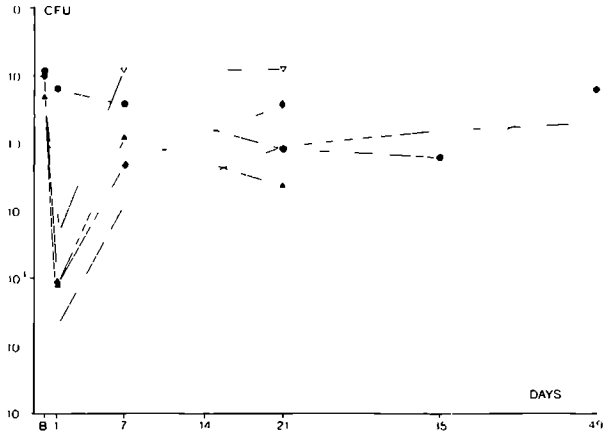

fig. 3 *S. sanguis* on smooth surfaces, same legend as fig. 1a.

The number of total Colony Forming Units was significantly suppressed 1 day after the chlorhexidine and SnF_2 treatments in fissures and on smooth surfaces ($P<0.01$, fig. 4) but not in approximal areas or in saliva. Chlorhexidine rinses (group IV) resulted in a small, not significant suppression of the total CFU in fissures and on smooth surfaces.

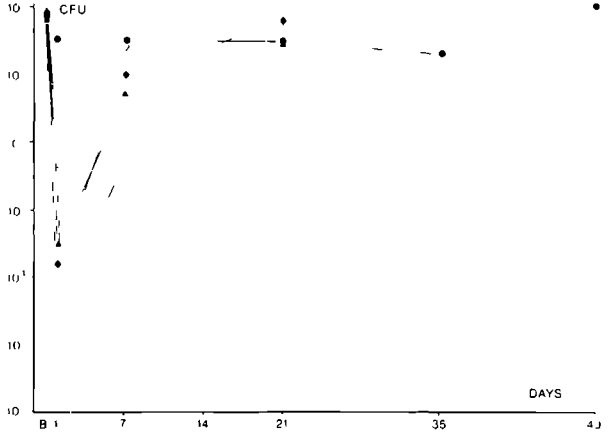

fig. 4 Total Colony Forming Units on smooth surfaces, same legend as fig. 1a.

The aim of this study was to suppress *S. mutans* by a short-term high dose application of chlorhexidine or stannous fluoride, and to study the kinetics of recolonization of the dentition by *S. mutans*, *S. sanguis* and *A. viscosus*.

The application of the chlorhexidine gel was based on a regime proposed by Maltz *et al.* (1981). We have condensed the treatment to a single session in order to reduce the treatment time and to make the treatment less demanding for the participants in the study. The stannous fluoride was applied in a conventional manner (Newbrun, 1978). In order to make the stannous fluoride treatment more readily comparable with the chlorhexidine treatment stannous fluoride was also applicated in triplo.

A convenient way to administer chlorhexidine would be to apply it as a dentifrice. Therefore we have incorporated such an experimental group in our study. After 7 days of brushing with chlorhexidine gel *S. mutans* was suppressed considerably less than after the local chlorhexidine applications. The moderate reduction of the *S. mutans* counts is possibly related to the concentration of the chlorhexidine gel and/or the time that the gel was in contact with the tooth surfaces. In a 12 months study of daily brushing with 0.5 chlorhexidine gel the observed *S. mutans* reductions were neither very impressive (Emilson and Fornell, 1976).

The strong initial suppression of *S. mutans* after chlorhexidine application was in accordance with the results of Emilson (1981), Maltz *et al.* (1981) and Zickert *et al.* (1982). Stannous fluoride application was found to be effective against *S. mutans* (Keene *et al.* 1977). Antimicrobial treatment of the dentition strongly suppressed the *S. mutans* counts on the mucosal surfaces. These surfaces are unlikely to act as *S. mutans* reservoirs for subsequent colonization of the dentition. The *S. mutans* counts in saliva were also lowered after chemotherapy and the reductions we measured are comparable with those observed by Maltz *et al.* (1981) and Zickert *et al.* (1982).

The strong suppression of *A. viscosus/naeslundii* after chemotherapy is in accordance with the results obtained by Yoon and Berry (1979) after SnF_2 application or after spot application of chlorhexidine in

our previous study (Schaeken *et al.*, 1984). Chlorhexidine caused a more longlasting suppression of *A. viscosus/naeslundii* than did SnF_2. Further, the treatment of the complete dentition suppressed *A. viscosus/naeslundii* for more than 7 days, whereas after spot application the original level was regained within 7 days after chemotherapy.

After chlorhexidine treatment the *S. sanguis* counts were found to increase proportionally (Emilson and Fornell, 1976; Emilson, 1981) or to remain unchanged (Schaeken *et al.* 1984). In this study we observed that 1 day after chlorhexidine or stannous fluoride application *S. sanguis* accounted for the majority of total CFU, but that compared with the baseline level or the control group the *S. sanguis* counts were strongly suppressed on smooth surfaces and in fissures. These observations are confirmed by the results obtained by Svanberg and Rolla (1982) after SnF_2 rinses.

The speed of colonization of the teeth by *S. mutans* is found to be related to the *S. mutans* concentration in saliva (Svanberg and Loesche, 1977; Duchin and van Houte, 1978). As the antimicrobial treatment of the dentition resulted in lower salivary counts, we expected a slower recolonization or a longer suppression of *S. mutans* on the dentition. Yet, it was found that the *S. mutans* population had reached the baseline level between 7 and 21 days after chemotherapy in fissures and approximal areas. Maltz and Zickert (1982) reported a similar short-term suppression of *S. mutans* after antimicrobial treatment with penicillin in man. The fast recolonization of *S. mutans* is probably related to the strong suppression of other bacteria. From clinical studies (Svanberg and Loesche, 1977; Schaeken *et al.*, 1984) and from animal experiments (van der Hoeven and Rogers, 1979; Beckers and van der Hoeven, 1982) it is known that the presence of oral bacteria, among which *A. viscosus* and *S. sanguis* (van der Hoeven, 1980) can retard or prevent the establishment of *S. mutans* by interference. *A. viscosus*, and to a lesser extend, *S. sanguis* were strongly suppressed after chemotherapy. The lower levels of *A. viscosus* on the dentition after chemotherapy might therefore facilitate the recolonization by *S. mutans*.

These results stress the need for antimicrobial agents that selectively suppress the target organism but not the other important species in the plaque flora.

When aselective agents such as chlorhexidine or stannous fluoride are used, the *S. mutans* levels after the local application can be kept down by using a low concentration chlorhexidine rinse or possibly a SnF_2 rinse (Svanberg and Rolla, 1982). While the chlorhexidine rinses remained effective against *S. mutans* and *A. viscosus* in fissures, the populations on smooth and approximal surfaces slowly recovered, although *S. mutans* was still significantly suppressed 5 weeks after the gel application. The use of dental floss is perhaps a better way to deliver antimicrobial agents in the approximal areas. Keene *et al.* (1977) applied SnF_2 in this manner and found that *S. mutans* could be suppressed effectively. For the use of dental floss some dexterity is required. The flossing was not succesful in one of the five subjects, probably by the improper way the floss was used at home. An alternative mode of bringing chlorhexidine in approximal areas is by using toothpicks. Kristoffersson and Bratthall (1982) observed that when approximal areas were exposed to chlorhexidine, applied by toothpicks 3 times for three minutes on two days, reductions in *S. mutans* counts could be observed for more than 40 days after the treatment.

The low concentrated chlorhexidine rinses resulted in a small (fissures and approximal areas) or moderate (smooth surfaces, saliva) reduction of the total CFU. These observations are in agreement with short term (Loe and Schiott 1970) and long term (Loe *et al.* 1976, Lang *et al* 1982) studies on the effect of regular application of low concentrated chlorhexidine solutions on the formation of dental plaque.

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VI-1 Summary

In the first part of chapter 1 a brief exposé is given on chemotherapy in caries prevention. It appears that for a small group of patients the conventional preventive and restorative treatment do not suffice in arresting caries. These high-caries risk patients need extra preventive help. Risk patients can be selected by microbiological tests such as the *Lactobacillus* counts and the *Streptococcus mutans* counts and by their initial smooth surface lesions. In preventive programs for risk subjects the use of fluoride compounds is of utmost importance. The additional caries prevention can come from food counselling and from antimicrobial therapy against *S. mutans*, as this organism is considered a major pathogen in caries in man.

The interaction of the fluoride ion with the tooth surface probably plays the crucial role in caries inhibition, while the inhibition of the bacterial metabolism by fluoride is probably of minor importance.

The antimicrobial treatment of cariogenic plaque may be carried out with concentrated fluoride compounds or antimicrobial agents. In several clinical studies chlorhexidine has proved to be a very potent antimicrobial agent. It has also been demonstrated in animal and human studies that by the combined use of chlorhexidine and fluoride more caries is inhibited than by fluoride alone.

In the second part of chapter 1 an outline of the experiments is given. The normal plaque microflora has a high resistance against colonization by alien organisms. Microbial species that play an important role in this colonization resistance are *Actinomyces viscosus* and *Streptococcus sanguis*. In a clinical study we therefore have tried to raise the colonization resistance of the dental plaque flora against *S. mutans* by deliberate application of high numbers of *A. viscosus* and *S. sanguis* on the teeth after chemotherapy. However, the application did not contribute to a fast re-establishment of the indigenous *A. viscosus/naeslundii* population. The experiments in chapter 2 were set up to find the reason for this negative result.

The dentition of rats was treated with a short-term high dose of chlorhexidine. At different time intervals after the chlorhexidine application *A. viscosus* was inoculated to investigate the effect of the

retained chlorhexidine on its establishment. When the *A. viscosus* strain was inoculated within 1 hour after the chlorhexidine treatment the colonization did not succeed. Inoculation after a longer time span resulted in a retardation of the initial colonization but eventually the *A. viscosus* population reached the normal level. In the clinical study an *A. viscosus* strain was implanted 5 hours after chlorhexidine application or after professional tooth cleaning. It was assumed that at 5 hours after application of chlorhexidine, the retained chlorhexidine would have only a bacteriostatic effect. The implanted *A. viscosus* strain colonized the dentition better after chlorhexidine application than after professional tooth cleaning, but still made up only 0.1-1 % of the total *A. viscosus/ naeslundii* population of the dentition.

As it seemed that inoculation of *A. viscosus* after chlorhexidine application did not stimulate the accumulation of the total *A. viscosus/naeslundii* population, we have directed subsequent experimentation to the effects of highly concentrated antimicrobial agents on *S. mutans* and the microbial composition of the dental plaque flora.

The experiments in chapter 3 describe the effects of short-term high dose therapies with chlorhexidine, iodine or 5,7-dichloro-8-hydroxyquinoline (DCHQ) on the microbial composition of dental plaque in conventional rats. The disinfectants were applied to the molars on 7 consecutive days. Smooth surface and fissure plaque samples were analysed 1, 3 and 10 days after termination of the chemotherapy. All disinfectants exerted a stronger effect on the microflora on smooth surfaces than in fissures. Chlorhexidine, but not iodine or DCHQ, eliminated *S. mutans* from plaque. Microbial species that were affected but not eliminated by chemotherapy were found to return rapidly to their original levels.

Chapter 4 deals with the effects of chlorhexidine and iodine on the composition of the human-dental plaque flora. Localized areas of the dentition in human volunteers were treated once with chlorhexidine or iodine. Plaque samples taken from the experimental surfaces were analysed for the number of *S. mutans*, *S. sanguis*, *A. viscosus* and the total viable counts. Chlorhexidine and iodine strongly suppressed *S. mutans* and *A. viscosus*, but *S. sanguis* was much less affected. *A. viscosus* returned to its original level within 7 days after

chemotherapy. *S. mutans* returned much slower to its pretreatment level. In fissures and restoration margins *S. mutans* was still significantly suppressed 21 days after chlorhexidine application.

In this clinical study the salivary *S. mutans* levels were not affected after the spot application of chlorhexidine and iodine. It has been found that the speed of recolonization of *S. mutans* is related to the salivary level of this bacterium (Svanberg and Loesche, 1977; Duchin and van Houte, 1978). The aim of the experiment described in chapter 5 was therefore to determine the effect of an intensive antimicrobial treatment of the entire dentition on the number of *S. mutans*, *S. sanguis*, *A. viscosus* and the total Colony Forming Units (CFU) in plaque.

The dentition of human volunteers was treated with either chlorhexidine or stannous fluoride. Following local treatment with chlorhexidine, selected volunteers rinsed daily for 7 or 49 days with chlorhexidine solution while another group flossed daily for 7 days with dental floss impregnated with chlorhexidine. On days 1, 7, 21, 35 and 49 after the local applications saliva samples and plaque samples from fissures, smooth surfaces and approximal areas, were taken.

Chlorhexidine and stannous fluoride suppressed *S. mutans* and *A. viscosus* on all surfaces and in saliva. *S. mutans* on tooth surfaces was suppressed for approximately 7 days and reached the baseline level at day 21. *A. viscosus* was suppressed for more than 7 days on the teeth. *S. sanguis* and the total CFU had regained the baseline level within 7 days on all surfaces and in saliva. Rinsing or flossing with chlorhexidine suppressed *S. mutans* for the time that these supplements were used.

Brushing for 7 days with chlorhexidine gel without a preceding intensive chlorhexidine treatment had virtually no effect on *S. mutans* on tooth surfaces or in saliva.

VI-2 Final conclusions and recommendations

The purpose of this study was to test some antimicrobial agents for their selective suppression of *S. mutans* and to try to retard the recolonization of *S. mutans* in human volunteers by bacterial species such as *A. viscosus*.

The experiments in chapter 2 showed that application of high numbers of *A. viscosus* on a surface, that was previously treated with chlorhexidine, does not contribute to a fast accumulation of *A. viscosus*. It was shown that the colonization of the *A. viscosus* strain was retarded by the chlorhexidine that remained on the surface after the antimicrobial treatment.

In chapter 3, 4 and 5 the effect of antimicrobial agents on the composition of the dental plaque microflora was studied under well-controlled conditions. Based on a survey of the literature, chlorhexidine, iodine and stannous fluoride were chosen for the clinical studies as these agents could exert a selective suppression on *S. mutans*. In rat experiments chlorhexidine was able to eliminate *S. mutans* from the dental plaque flora (chapter 3). In addition to *S. mutans*, *A. viscosus* was strongly suppressed, for a short period of time. The latter bacterial species plays an important role in the colonization resistance of the dental plaque flora against *S. mutans*. After disinfection of an area infected with *S. mutans*, the area is colonized by *S. sanguis* and, after a short period of time, also by *A. viscosus*. When the *S. mutans* counts in saliva are not too high the newly formed plaque with *S. sanguis* and *A. viscosus* retards the colonization by *S. mutans* for more than 3 weeks. In this way chlorhexidine acts quite selective against *S. mutans* (chapter 4). After disinfection of the complete dentition with chlorhexidine the *A. viscosus* numbers in saliva are also suppressed. As a result, *S. mutans* is able to colonize the newly formed plaque, that consists mainly of *S. sanguis*, more readily. Therefore, chlorhexidine acts less selective against *S. mutans* after treatment of the entire dentition (chapter 5).

The results from these experiments stress the need for agents with a selective action against *S. mutans*.

From the fact that a short-term intensive treatment with chlorhexidine or stannous fluoride failed to result in a long-term suppression of *S. mutans*, it cannot be concluded that the antimicrobial treatment with these agents is useless. In clinical trials the combination of fluoride and chlorhexidine was shown to better prevent caries than fluoride did (a short review is given in chapter 1). Therefore it is advised to treat caries-risk patients with fluoride and, in addition, with antimicrobial agents against *S. mutans*. Patients are considered to have a high risk for caries when they have high salivary number of *S. mutans* (more than $2.5 \cdot 10^5$ CFU per ml (Zickert *et al.*, 1982), of lactobacilli (more than 10^5 CFU per ml), and a high number of initial smooth surface lesions. Also patients in which the caries progression is considerably enhanced by orthodontic or prosthetic devices, and patients that have undergone periodontal flap operations or root planing have a higher risk for caries.

A preventive regime that has been shown to be effective in risk patients includes the application of a gel (pH 7.2) consisting of 1 % chlorhexidine digluconate (ICI, England) in 4 % methylcellulose. The patient is instructed to apply the gel 5 min daily for 14 days in prefabricated trays (Centrays®, Pacemaker Corp.) or individual trays. Prior to the antimicrobial treatment the dentition is cleaned and defective restorations are repaired. The carious lesions are repaired after the antimicrobial therapy has been finished. When after 4 months the *S. mutans* counts in saliva exceed $2.5 \cdot 10^5$ CFU per ml, the treatment is repeated (Zickert *et al.*, 1982).

Based on our own experience a less elaborate but more intensive treatment is likely to give good results. Such a treatment could exist of a dental prophylaxis, including cleaning of the interproximal areas with dental floss impregnated with chlorhexidine (unwaxed dental floss soaked for 24 hours in a 5 % chlorhexidine solution and subsequently dried). Following prophylaxis a 2.5 % chlorhexidine gel is applicated three times for 5 minutes using applicator trays and taking an interval between the treatments of approximately 5 minutes.

If necessary, *S. mutans* can be strongly suppressed for a prolonged period of time by a local chlorhexidine treatment followed by daily rinses with a 0.2 % chlorhexidine solution (e.g. Hibident®, ICI). This long-term chemotherapy is indicated in patients with caries susceptible

dentitions that require caries promoting devices as splints after tooth- or jaw fractures and patients that temporarily wear removable prosthetic devices while they are treated for extensive occlusal rehabilitations.

Finally, it can be considered to combine the antimicrobial regimes with the application of fluoride by incorporating 0.1 % sodium fluoride (NaF) in the gel or 0.05 % NaF in the solution. The pH of the gel or solution must be neutral as chlorhexidine is not effective at low pH.

Literature: vide chapter 1

VII-1 Samenvatting

Ondanks de dalende cariesprevalentie blijkt dat bij een relatief kleine groep patienten de conventionele preventieve en restauratieve hulp niet voldoende is om cariës tot staan te brengen. Een verhoogd risico op cariës ontstaat bij patienten waarbij de speekselvloed is verminderd - b.v. door medicijngebruik of na bestraling van de speekselklieren -, bij patienten met orthodontische of prothetische voorzieningen, bij frequent suikergebruik en een cariogene plaqueflora. *Streptococcus mutans* wordt beschouwd als het belangrijkste pathogene microorganisme bij glazuurcaries. Hoge aantallen *S. mutans* in de tandplaque op een bepaalde plaats van een gebitselement gaan vaak gepaard met een carieuze lesie op die plaats (Shklair *et al.*, 1972; Loesche *et al.*, 1975a; Burt *et al.*, 1983). Ook is gebleken dat bij individuen met een hoge cariesactiviteit meer plaatsen op het gebit besmet zijn met *S. mutans* dan bij individuen met een lage cariesactiviteit (Gibbons *et al.*, 1974), terwijl personen met meer dan 10^6 *S. mutans*-cellen per ml speeksel meer carieuze lesies ontwikkelen dan personen met lagere aantallen *S. mutans* (Klock en Krasse, 1979, Zickert *et al.*, 1983). Op grond van de relatie van *S. mutans* met caries zijn verschillende microbiologische tests ontwikkeld teneinde patienten met een verhoogd risico op cariës te selecteren (van der Hoeven, 1981; Newbrun, 1983). In de praktijk komen deze tests er op neer dat de aantallen *S. mutans* in het speeksel of op een representatief gebitsoppervlak geteld worden. Momenteel zijn er nog geen *S. mutans*-tests ontwikkeld voor gebruik in de algemene tandartspraktijk, zodat voor het verwerken van de plaque of speekselmonsters een bacteriologisch laboratorium ingeschakeld moet worden. Risicogroepen kunnen eventueel ook geselecteerd worden aan de hand van de aantallen lactobacillen in het speeksel. Het blijkt namelijk dat bij patiënten zonder al te veel retentieplaatsen (open caviteiten, orthodontische of prothetische voorzieningen), de hoeveelheid suiker die geconsumeerd wordt goed correleert met het aantal *Lactobacillus*-cellen in het speeksel. Hoge aantallen lactobacillen, meer dan 10^5 per ml speeksel, duiden op een hoog suikergebruik. Voor de telling van het aantal lactobacillen in het speeksel is een test

ontwikkeld, die min of meer geschikt is voor gebruik in de algemene praktijk (Dentocult®, Orion Diagnostica, Helsinki, Finland; Larmas, 1975).

De microbiologische cariesactiviteitstests zijn behept met een zekere fout: circa 20 % van de personen met een hoog aantal van de betrokken bacteriesoort (*S. mutans* of *Lactobacillus* species) ontwikkelt geen caries, terwijl omgekeerd 20 % van de personen met een laag aantal niet vrij blijft van nieuwe carieuze lesies (Krasse, 1976; Crossner, 1981). De voorspellende waarde van deze tests kan echter verhoogd worden door ook rekening te houden met het aantal beginnende carieuze lesies (Klock en Krasse, 1979).

Caries kan in principe voorkómen worden met een weinig cariogeen voedingspatroon, door het gebit resistenter te maken tegen de bacteriele zuren en door de cariogene microflora in een niet-cariogene te veranderen. Bij de behandeling van risicopatienten staat het gebruik van fluoride centraal. De anticariogene werking van fluoride berust grotendeels op het minder oplosbaar maken van het glazuur. Caries wordt bevorderd door een frequent en hoog suikergebruik. Het veranderen van zo een cariogeen voedingspatroon blijkt in de praktijk moeilijk te zijn. De tandarts kan de patient hierbij helpen door bij voorbeeld de lactobacillen-aantallen als graadmeter voor het suikergebruik te nemen (Krasse, 1976).

Het veranderen van een cariogene plaqueflora in een niet-cariogene kan in principe plaatsvinden door selectieve onderdrukking van niet-gewenste bacteriesoorten. Voor deze chemotherapie heeft men in klinische onderzoeken een groot aantal agentia gebruikt zoals antibiotica, desinfectantia en geconcentreerde fluoridepreparaten (voor een overzicht, zie Loesche, 1982). Omdat *S. mutans* de belangrijkste pathogeen is bij glazuurcaries is de antimicrobiele behandeling er meestal op gericht deze bacteriesoort uit de plaque te verdrijven of tot lagere aantallen terug te brengen. De laatste jaren is daarom vooral met chloorhexidine, jodium en tinfluoride onderzoek gedaan, omdat deze middelen *S. mutans* selectief zouden onderdrukken. Hierbij werd gevonden dat een kortdurende intensieve behandeling met deze stoffen *S. mutans* sterk onderdrukt. Deze onderdrukking is echter slechts tijdelijk en na verloop van enkele weken heeft de *S. mutans*-populatie weer zijn oude niveau bereikt.

Uit klinisch en dierexperimenteel onderzoek is bekend dat de vestiging van *S. mutans* op het gebit geremd of zelfs verhinderd kan worden door andere bacteriesoorten in de tandplaque (van der Hoeven, 1980). Svanberg en Loesche (1977) brachten bij proefpersonen artificiele fissuren aan en zagen dat wanneer de fissuur gevuld was met mondbacterien, *S. mutans* zich niet meer kon vestigen, terwijl lege fissuren binnen een dag door *S. mutans* gecoloniseerd werden. In dierexperimenten van van der Hoeven en Rogers werd de plaqueflora van ratten aangevuld met *Actinomyces viscosus* en *Streptococcus sanguis*, twee veel voorkomende mondbacterien. Het bleek dat het voor *S. mutans* veel moeilijker werd om deze ratten te coloniseren, met name wanneer *A. viscosus* en *S. sanguis* zich enige tijd hadden kunnen stabiliseren (voor een overzicht van deze experimenten zie: van der Hoeven, 1980).

Op deze waarnemingen is een klinisch experiment gebaseerd, waarin wij de hercolonisatie van *S. mutans* hebben getracht te verhinderen door direkt na de behandeling met chloorhexidine hoge aantallen *A. viscosus* op de behandelde vlakken te enten. Het bleek echter dat de toename van de *A. viscosus*-populatie in de proefpersonen bij wie wij *A. viscosus* op de tanden hadden aangebracht hetzelfde was als in proefpersonen bij wie *A. viscosus* niet aangebracht was (niet gepubliceerde resultaten). Om de oorzaak van dit teleurstellende resultaat te onderzoeken zijn de experimenten verricht, die in hoofdstuk 2 beschreven staan. Chloorhexidine bezit *in vivo* een sterke antimicrobiele werking die mogelijk samenhangt met de adsorptie van deze stof aan het gebit en aan de slijmvliezen. Na adsorptie zou chloorhexidine gedurende langere tijd vrij kunnen komen in bacteriostatische concentraties. Om te onderzoeken of dit vermoeden juist was werd het dierexperiment in hoofdstuk 2 verricht. Het gebit van ratten werd kortdurend behandeld met een hoge dosis chloorhexidine. Op verschillende tijdstippen na chloorhexidine-applicatie werd *A. viscosus* in de mond aangebracht. Wanneer dit gebeurde binnen 1 uur na beeindiging van de chloorhexidine-applicatie dan vestigde de *A. viscosus* stam zich meestal niet. Bij inoculatie van *A. viscosus* op een later tijdstip werd wel de initiele colonisatie geremd, doch groeide de *A. viscosus*-populatie uiteindelijk tot het normale aantal uit. Bij het klinisch onderzoek in hoofdstuk 2 werd getracht bij proefpersonen hoge aantallen van een *A. viscosus* stam op het gebit te laten vestigen door deze 5 uur na de behandeling in de

mond aan te brengen. Het bleek dat de *A. viscosus* stam het gebit beter coloniseerde na chloorhexidine-applicatie dan na alleen gebitsreiniging, doch slechts 0,1-1 % van de totale *A. viscosus/naeslandii*-populatie uitmaakte. Waarschijnlijk heeft het chloorhexidine dat op het vlak achtergebleven was de vestiging van de *A. viscosus*-stam belemmerd.

Naar het *in vivo* effekt van antimicrobiele middelen als chloorhexidine op andere bacterien dan *S. mutans* is weinig onderzoek verricht. Om onder gecontroleerde omstandigheden het effekt van een aantal agentia op de microflora te bestuderen werden de experimenten in hoofdstuk 3, 4 en 5 uitgevoerd.

Bij de experimenten in hoofdstuk 3 werd onderzocht hoe de microbiele samenstelling van de tandplaque van conventionele ratten veranderde na kortdurende intensieve chemotherapie met chloorhexidine, jodium en 5,7-dichloro-8-hydroxyquinoline, DCHQ. Uit klinisch onderzoek is bekend dat chloorhexidine en jodium (Caufield en Gibbons, 1979) *S. mutans* in de tandplaque sterk onderdrukken. DCHQ is een stof die *in vitro S. mutans* sterk remt doch weinig effekt heeft op *A. viscosus* (Tanzer *et al.*, 1978). De desinfectantia werden op 7 opeenvolgende dagen geappliceerd. Plaque-monsters van gladde vlakken en fissuren werden geanalyseerd op 1, 3 en 10 dagen na beeindiging van de chemotherapie.
Alle desinfectantia werkten beter op de gladde vlakken dan in de fissuren. Chloorhexidine elimineerde *S. mutans* uit de tandplaque doch bij jodium en DCHQ was dit niet het geval. Bacteriesoorten die wel onderdrukt werden doch niet geelimineerd bleken na stopzetten van de behandeling weer snel tot hun oorspronkelijke aantal uit te groeien.

In hoofdstuk 4 wordt het effekt van chloorhexidine en jodium op de samenstelling van de humane tandplaqueflora nagegaan. Goed omschreven plaatsen op het gebit van vrijwilligers werden eenmalig met chloorhexidine of jodium behandeld. Plaquemonsters van de experimentele vlakken werden na 2, 7 en 21 dagen genomen en onderzocht op de aantallen *S. mutans*, *S. sanguis*, *A. viscosus* en het totaal aantal kweekbare bacterien. Chloorhexidine en jodium onderdrukten *S. mutans* en *A. viscosus* sterk, terwijl *S. sanguis* veel minder geremd werd. De *A. viscosus*-populatie had na 7 dagen het oorspronkelijke aantal weer bereikt, terwijl *S. mutans* het beginniveau veel langzamer bereikte. In fissuren en vullingsranden was *S. mutans* 21 dagen na de chloorhexi-

dine-applicatie nog significant onderdrukt.
De recolonisatie van *S. mutans* verliep het snelste bij proefpersonen met hoge aantallen *S. mutans* in het speeksel. Omdat in dit onderzoek slechts een klein gedeelte van het gebit behandeld werd met chloorhexidine of jodium veranderde de bacteriele samenstelling van het speeksel niet.

In de experimenten die in hoofdstuk 5 beschreven worden, werd getracht door antimicrobiële behandeling van het gehele gebit de *S. mutans*-aantallen in het speeksel te verlagen en aldus de recolonisatie van *S. mutans* te vertragen. Het gehele gebit van proefpersonen werd behandeld met chloorhexidine of met tinfluoride. Na de locale applicatie spoelden twee groepen proefpersonen respectievelijk 7 en 49 dagen met een chloorhexidine-oplossing, terwijl een andere groep proefpersonen 7 dagen met chloorhexidine geïmpregneerde floss gebruikte.
Op dag 1, 7, 21, 35 en 49 na de locale applicatie werden speeksel en plaquemonsters genomen van gladde en approximale vlakken en uit fissuren. In de monsters werden de aantallen *S. mutans*, *S. sanguis*, *A. viscosus* en het totaal aantal kweekbare bacterien geteld.
Chloorhexidine en tinfluoride onderdrukten *S. mutans* en *A. viscosus* op alle gebitsoppervlakken en in het speeksel. *S. mutans* werd in de tandplaque ongeveer 7 dagen significant onderdrukt en was na 21 dagen weer tot het oorspronkelijke aantal uitgegroeid. *A. viscosus* werd in de tandplaque meer dan 7 dagen onderdrukt. Het aantal *S. sanguis* en het totaal aantal kweekbare bacterien was binnen 7 dagen weer tot het oorspronkelijke aantal teruggekeerd. Spoelen of flossen met chloorhexidine onderdrukte *S. mutans* gedurende de tijd dat deze middelen gebruikt werden.

VII-2 Eindconclusie en aanbevelingen

In dit onderzoek heb ik willen nagaan wat de mogelijkheden zijn om *S. mutans* selectief te onderdrukken en de hercolonisatie van deze bacterie vervolgens te belemmeren met behulp van andere bacterien zoals *A. viscosus*.

In de experimenten uit hoofdstuk 2 bleek dat het aanbrengen van hoge aantallen *A. viscosus* na behandeling van een gebitsoppervlak met chloorhexidine niet leidt tot een snelle accumulatie van *A. viscosus*, maar dat de colonisatie van de *A. viscosus*-stam geremd wordt door chloorhexidine dat op het gebitsoppervlak is achtergebleven.

In de hoofdstukken 3, 4 en 5 werd het effekt van enkele antimicrobiele middelen op de bacteriele samenstelling van de tandplaque onder gecontroleerde omstandigheden bestudeerd. De keuze van deze agentia was gebaseerd op gegevens uit de literatuur. Omdat chloorhexidine, jodium en tinfluoride *S. mutans* selectief zouden onderdrukken hebben wij deze middelen gekozen.

In dierexperimenten bleek dat chloorhexidine in staat is *S. mutans* uit de plaque te elimineren (hoofdstuk 3). Naast *S. mutans* werd ook *A. viscosus* kortdurend sterk onderdrukt. Deze bacteriesoort, die doorgaans zeer belangrijk is in de supragingivale plaque, speelt een belangrijke rol bij de colonisatieresistentie van de plaquemicroflora tegen *S. mutans*. Na desinfectie van een met *S. mutans* besmette plaats kunnen *S. sanguis*, en korte tijd later ook *A. viscosus* zich vanuit het speeksel op deze gedesinfecteerde plaats vestigen. Wanneer de aantallen *S. mutans* in het speeksel niet te hoog zijn dan kan de nieuw gevormde plaque met *A. viscosus* en *S. sanguis* de colonisatie van *S. mutans* gedurende enige weken sterk remmen. Chloorhexidine werkt in dit geval redelijk selectief (hoofdstuk 4).

Na desinfectie van het gehele gebit met chloorhexidine worden ook de *A. viscosus*-aantallen in het speeksel en in de plaque sterk onderdrukt. Het gevolg is dat *S. mutans* de nieuw gevormde plaque die in het begin grotendeels uit *S. sanguis* bestaat gemakkelijker kan coloniseren. Na behandeling van het gehele gebit met chloorhexidine werkt deze stof dan ook minder selectief tegen *S. mutans* dan na plaatselijke applicatie (hoofdstuk 5).

De resultaten van dit onderzoek benadrukken de behoefte aan middelen met een grotere selectiviteit ten aanzien van *S. mutans*.

Uit het feit dat *S. mutans* niet langdurig onderdrukt kan worden met middelen als chloorhexidine of tinfluoride mag niet geconcludeerd worden dat de antimicrobiele behandeling met deze stoffen zinloos is. Uit klinisch onderzoek bij caries risicopatienten is immers gebleken dat fluoride gecombineerd met chloorhexidine meer caries voorkomt dan fluoride alleen (voor een kort overzicht, zie hoofdstuk 1) Het is daarom aan te bevelen deze risicopatiënten naast fluoride ook met antimicrobiele middelen tegen *S. mutans* te behandelen. De diagnose "risicopatient" kan gesteld worden op grond van hoge aantallen *S. mutans* (meer dan $2.5 \cdot 10^5$ CFU per ml speeksel, Zickert *et al.*, 1982), van lactobacillen (meer dan 10^5 CFU per ml speeksel), gecombineerd met gegevens over de cariesactiviteit (het aantal beginnende carieuze lesies). Tot de cariesrisicogroep behoren ook patienten waarin de cariesprogressie sterk verhoogd is door orthodontische of prothetische voorzieningen en patienten die parodontaal chirurgische ingrepen als "flaps" of "rootplaning" ondergaan hebben.

Een preventief regime dat in klinisch onderzoek effectief gebleken is bij risicopatiënten houdt in het appliceren van een gel (pH 7,2) bestaande uit 1 % chloorhexidine digluconaat (ICI, Engeland) in 4 % methylcellulose, waarvan de smaak gecorrigeerd is met enkele druppels pepermuntolie (Zickert *et al.*, 1982) De gel wordt gedurende 14 dagen 5 minuten per dag geappliceerd in geprefabriceerde trays (Centrays©, Pacemaker Corp) of individuele trays. De patient wordt aldus geïnstrueerd door de tandarts. Voorafgaand aan de antimicrobiele behandeling wordt het gebit gereinigd van tandsteen en worden defecte vullingen bijgewerkt. Na de behandeling worden de carieuze lesies gerestaureerd. Als na 4 maanden de *S. mutans*-aantallen hoger dan $2.5 \cdot 10^5$ CFU per ml speeksel zijn dan wordt de behandeling herhaald. Op grond van onze bevindingen zou een kortere maar intensievere behandeling ook tot goede resultaten kunnen leiden. Zo'n behandeling zou kunnen bestaan uit een gebitsreiniging, waarbij de interdentale gebieden gereinigd worden met dental floss geïmpregneerd met chloorhexidine (24 uur in 5 % chloorhexidine-oplossing dompelen en daarna laten drogen). Vervolgens wordt drie keer gedurende 5 minuten

een 2,5 % chloorhexidinegel geappliceerd in trays. Tussen elke behandeling is een rustperiode van circa 5 minuten.

Indien het noodzakelijk is dat de *S. mutans*-aantallen gedurende langere tijd sterk onderdrukt blijven, kan men de patient na de lokaalapplicatie dagelijks laten spoelen met een 0,2 % chloorhexidine-oplossing (b.v. Hibident®, ICI). Hiervoor komen in aanmerking patienten met cariesgevoelige gebitten, waar voor het gebit belastende voorzieningen aangebracht moeten worden als spalken na tand- of kaakfracturen of patienten die in afwachting van uitgebreide occlusale rehabilitaties partiele plaatprotheses dragen.

Tenslotte kan men overwegen om de antimicrobiele regimes te kombineren met applicatie van fluoride door in de gel of de spoelvloeistof resp. 0,1 % en 0,05 % natriumfluoride bij te mengen. De pH van de gel of vloeistof moet neutraal blijven, omdat chloorhexidine bij lage pH zijn werkzaamheid verliest.

Literatuur: zie hoofdstuk 1.

Curriculum Vitae

Geboortedatum en -plaats: 30 april 1952 te Weert.

Opleidingen: Mulo-B te Weert (eindexamen, juni 1969); voorbereidend jaar en Hogere Technische School voor Natuurkunde te Eindhoven (eindexamen, juni 1974), Colloquium Doctum en studierichting Tandheelkunde aan de Katholieke Universiteit te Nijmegen (doctoraalexamen, augustus 1978; tandartsexamen, maart 1980).

Van 1 december 1979 tot 1 november 1983 ben ik part-time werkzaam geweest op het laboratorium voor Orale Microbiologie van de afdeling Preventieve en Sociale Tandheelkunde, K.U. Nijmegen. Het proefschrift is gebaseerd op onderzoek dat in deze periode verricht is.

Na mijn tandartsexamen heb ik op enkele korte onderbrekingen na als medewerker in twee algemene praktijk gewerkt. Sinds december 1983 werk ik bij de Stichting Jeugdtandverzorging Oostelijk Gelderland.

Vanaf 1 januari 1984 ben ik part-time verbonden aan het instituut voor Occlusie Opbouw, subfaculteit Tandheelkunde, K.U. Nijmegen om onderzoek te doen naar de etiologie en preventie van tandwortelcaries.

STELLINGEN

I Voor een succesvolle onderdrukking van specifieke pathogene microorganismen in de tandplaque dient de colonisatieresistentie van de tandplaque tegen deze microorganismen zoveel mogelijk intakt te blijven.

(dit proefschrift)

II *Streptococcus mutans* kan gedurende langere tijd in de tandplaque worden onderdrukt wanneer na chemotherapie dagelijks met chloorhexidine wordt gespoeld.

(dit proefschrift)

III Het mechanisme dat Loesche voorstelt om te verklaren hoe *Streptococcus mutans* na een kortdurende "desinfektie" van het gebitsoppervlak uit de tandplaque zou worden verwijderd is onjuist. Daarbij wordt geen rekening gehouden met de bakteriele successie die optreedt na de initiele hechting van bakteriën op het behandelde oppervlak.

(Loesche: Dental Caries, 1983)

(dit proefschrift)

IV Cariës en parodontitis zijn geen continu voortschrijdende aandoeningen, doch kenmerken zich door plotselinge toenames en periodes van herstel.

V Klinisch onderzoek naar de preventie en behandeling van cariës en parodontitis dient verricht te worden bij patienten waarbij deze aandoeningen in een aktieve vorm aanwezig zijn. Om deze patiënten te selekteren kan men microbiologische parameters gebruiken.

VI De Nederlandse tandartsen zouden ertoe over moeten gaan de mogelijkheden van de *Streptococcus mutans*-telling en de daaraan gekoppelde chemotherapie te gebruiken.

VII Gezien het feit dat in de tandplaque zowel obligaat aerobe als obligaat anaerobe microorganismen voorkomen, is het merkwaardig, dat maar zo weinig aandacht is besteed aan zuurstof als ecologische faktor in de tandplaque.

VIII De veronderstelde pathogeniciteit van *Actinomyces viscosus* bij cariës van het tandwortelоppervlak en bij parodontitis is onvoldoende gefundeerd.

IX Sonderen van carieuze lesies is wat betreft de cariësdiagnostiek overbodig en kan schadelijk zijn voor de patiënt.

X De recente maatregelen van de Vereniging van Nederlandse Ziekenfondsen en de Nederlandse Maatschappij tot bevordering der Tandheelkunde komen de tandheelkundige verzorging van de ziekenfondspatiënten niet ten goede.

XI The tragic removal of the heart of Nijmegen during World War II afforded Dutch architects and planners an opportunity which they largely failed to measure up to.

(naar: T.M. Brown: The work of G. Rietveld architect, proefschrift Utrecht 1958, stelling VIII).

XII Alle architecten die vandaag de dag internationaal beroemd zijn, zijn dat door de stoelen die zij ontworpen hebben. De geschiedenis van de architectuur komt overeen met die van de moderne stoel.

(Ton Pompert: Triomf der Architectuur 1983/1984)

XIII Conceptuele kunst bestaat niet.

M.J.M. Schaeken

Nijmegen, 10 mei 1984